FOURTH REPORT

WELLCOME TROPICAL RESEARCH LABORATORIES

AT THE

GORDON MEMORIAL COLLEGE

KHARTOUM

ANDREW BALFOUR, M.D.

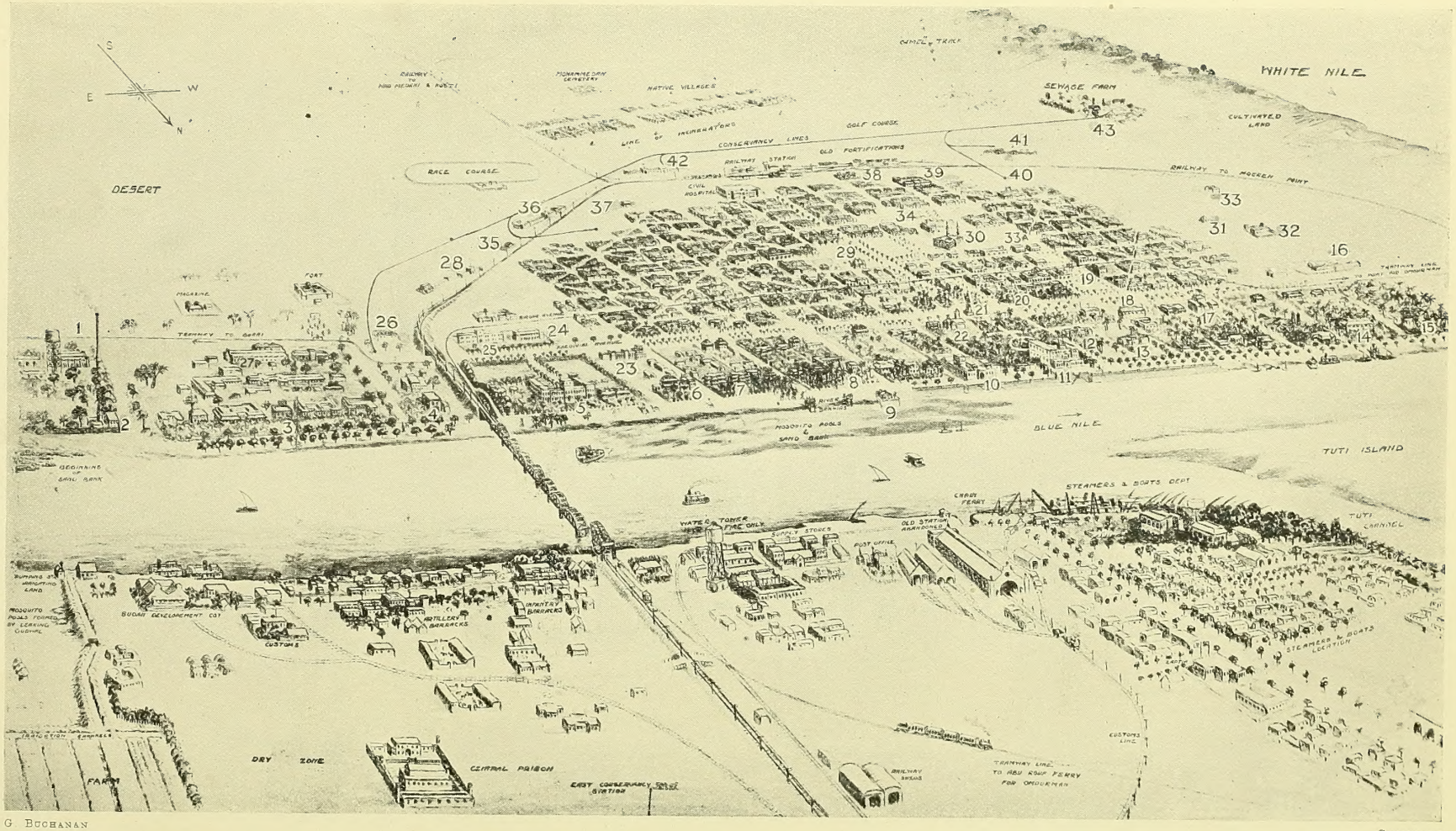
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Fig. 1.—Relief Map of Khartoum and a part of Khartoum North

Representing an area of three miles from East to West, and three miles from North to South

(Vide opposite page for detailed notes)

RELIEF MAP OF KHARTOUM AND A PART OF KHARTOUM NORTH

showing especially the following items of sanitary interest:—

IN KHARTOUM.—Ice factory, waterworks, sakias for garden irrigation, Laboratory steamer *Culex* and Floating Laboratory close to mosquito pools in river sandbank (these latter are usually much more numerous at Buri above the town), slaughter-house, meat and fish markets, conservancy tramway and collecting stations, sewage farm with refuse destructor, small incinerators serving native villages in the desert.

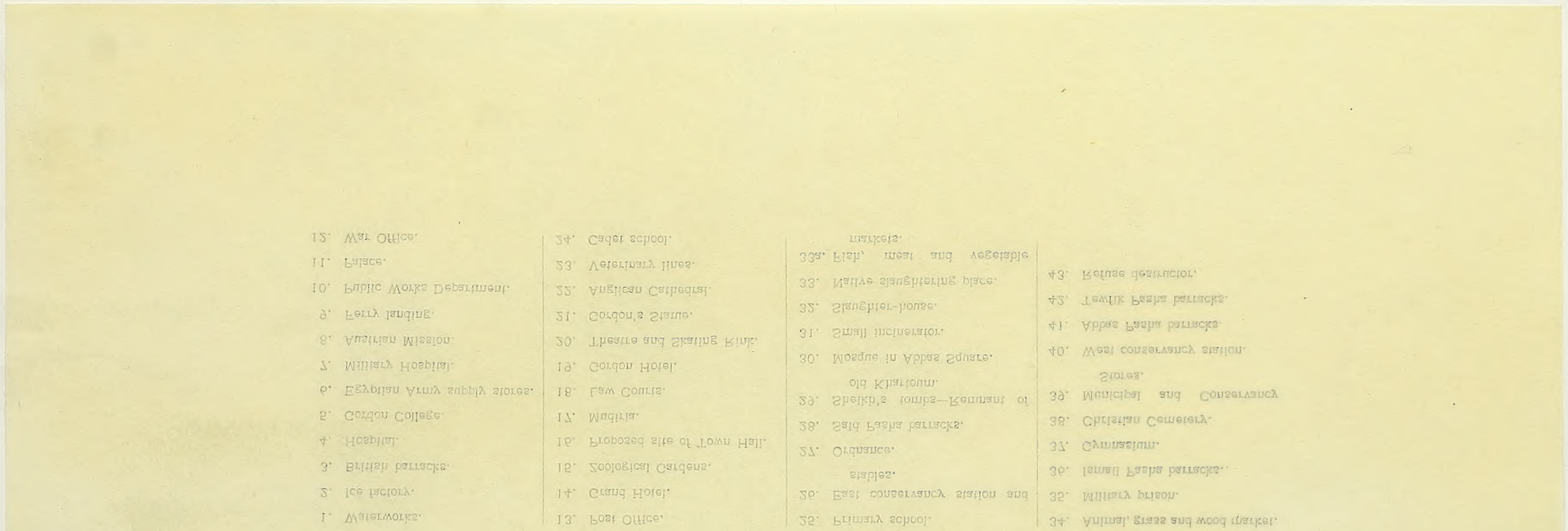
IN KHARTOUM NORTH.—Bold river bank in which mosquito pools do not form, portion of area of irrigated land one source of Anopheline mosquitoes, limited dry zone, conservancy collecting stations, dockyard where steamers and boats harbouring adult mosquitoes or their developmental stages are now but rarely found, though at one time common.

Only a small portion of the large native locations, harbouring thousands of people, is shown. These are served both by public latrines and by small incinerators, and the type of dwelling and general arrangement can be seen by reference to Fig. 93.

The drawing represents an area of three miles from East to West, and of three miles from North to South.

LIST OF BUILDINGS, ETC.

1. Waterworks.
2. Ice factory.
3. British barracks.
4. Hospital.
5. Gordon College.
6. Egyptian Army supply stores.
7. Military Hospital.
8. Austrian Mission.
9. Ferry landing.
10. Public Works Department.
11. Palace.
12. War Office.
13. Post Office.
14. Grand Hotel.
15. Zoological Gardens.
16. Proposed site of Town Hall.
17. Mudiria.
18. Law Courts.
19. Gordon Hotel.
20. Theatre and Skating Rink.
21. Gordon's Statue.
22. Anglican Cathedral.
23. Veterinary lines.
24. Cadet school.
25. Primary school.
26. East conservancy station and stables.
27. Ordnance.
28. Said Pasha barracks.
29. Sheikh's tombs—Remnant of old Khartoum.
30. Mosque in Abbas Square.
31. Small incinerator.
32. Slaughter-house.
33. Native slaughtering place.
33a. Fish, meat and vegetable markets.
34. Animal, grass and wood market.
35. Military prison.
36. Ismail Pasha barracks.
37. Gymnasium.
38. Christian Cemetery.
39. Municipal and Conservancy Stores.
40. West conservancy station.
41. Abbas Pasha barracks.
42. Tewfik Pasha barracks.
43. Refuse destructor.



KEPLEY MAP OF KHARTOUM AND A PART OF KHARTOUM NORTH

showing especially the following items of sanitary interest:—

IN KHARTOUM.—Ice factory, waterworks, sheds for Sudan Irrigation Laboratory, Arsenal, Camel and Frontier Laboratory close to mosquito boots in front; sandpark (these latter are usually much more unhealthy at Bait above the town); slaughter-house, meat and fish markets, cemetery, tramway and collective stations, sewage farm with refuse destructor, small factories serving native villages in the desert.

IN KHARTOUM NORTH.—Bold type part in which mosquito boots do not form, portion of area of interest land one source of Anopheline mosquitoes; limited qty zone, conservancy collective stations, dockyard where steamers and posts Metropolitan white mosquitoes or their developmental stages are now put rarely found, though at one time common.

Only a small portion of the large native locations, metropolitan thousands of people, is shown. These are served both by public latrines and by small incinerators, and the type of qualline and general arrangement can be seen by reference to Fig. 23.

The diagram represents an area of three miles from East to West, and of three miles from North to South.

List of Buildings, etc.

1. Waterworks.	13. Post Office.	25. Egyptian conservancy station and stables.	34. Animal, grass and wood market.
2. Ice factory.	14. Grand Hotel.	26. Egyptian Army supply stores.	35. Arabian Cafe.
3. British barracks.	15. Zoological Gardens.	27. Orphanage.	36. Native Cafe.
4. Hospital.	16. Proposed site of Town Hall.	28. Arab Cafe barracks.	37. Cemetery.
5. Cotton College.	17. Mudirie.	29. Sheikh's house—Kennant of old Khartoum.	38. Christian Cemetery.
6. Egyptian Army supply stores.	18. Law Courts.	30. Mosque in Arabs Square.	39. Municipal and Conservancy Stores.
7. Military Hospital.	19. Cotton Hotel.	31. Small factories.	40. West conservancy station.
8. Austrian Mission.	20. Theatre and Sharing Club.	32. Primary school.	41. Arabs Cafe barracks.
9. Ferry landing.	21. Cotton's Siding.	33. Native slaughter place.	42. Tewfik Cafe barracks.
10. Public Works Department.	22. Austrian Cathedral.	33a. Fish, meat and vegetable markets.	43. Native destructor.
11. Prison.	23. Veterinary lines.		
12. Customs.	24. Cadet school.		

The diagram represents an area of three miles from East to West, and of three miles from North to South.

FOURTH REPORT

OF THE

WELLCOME
TROPICAL RESEARCH LABORATORIES

AT THE

GORDON MEMORIAL COLLEGE
KHARTOUM

VOLUME A.—Medical

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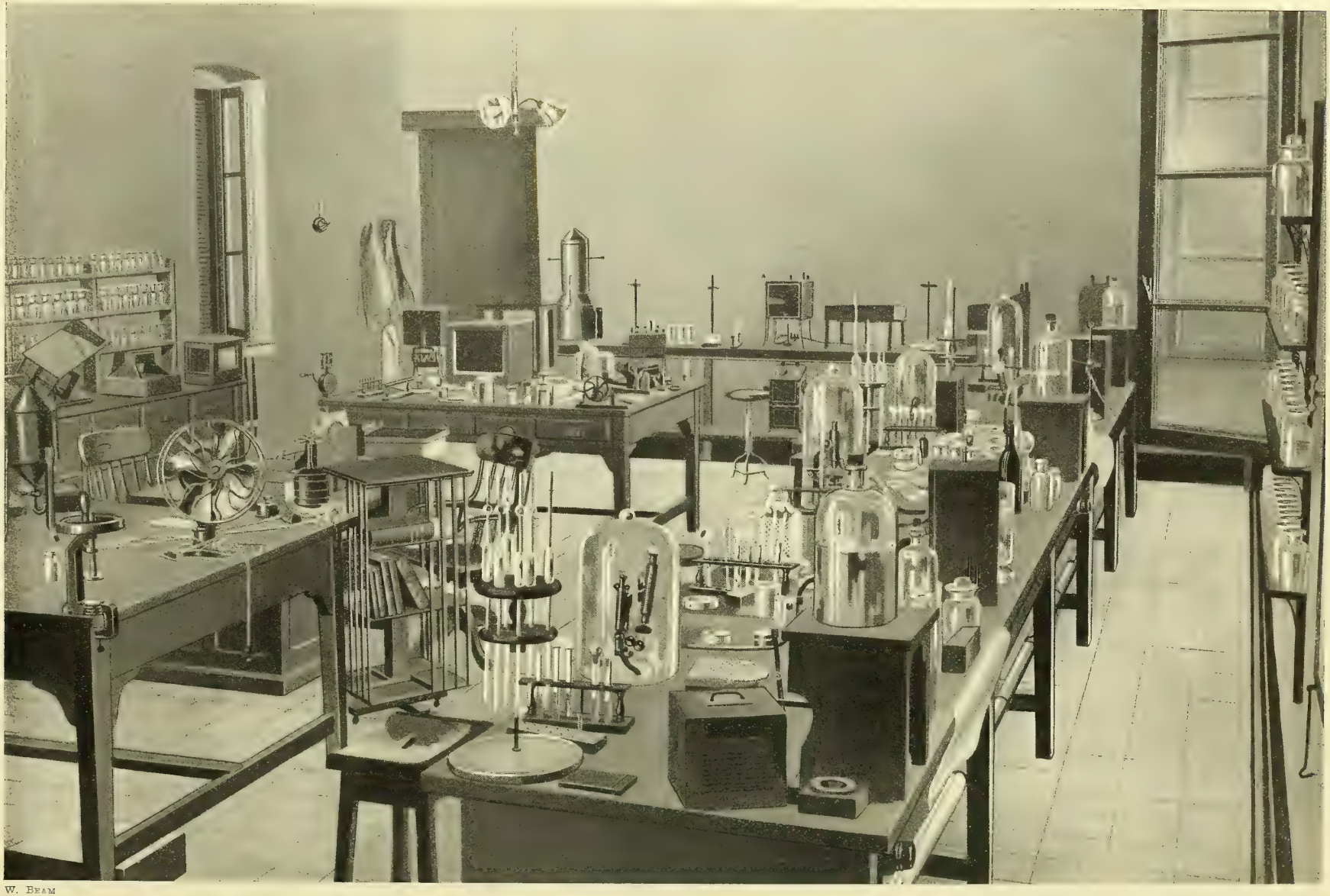
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W. Beam

Fig. 2.—Sectional View of the Bacteriological Laboratory

INTRODUCTION

A LONGER time than usual has, on this occasion, elapsed between the appearance of Reports. This is due in part to the fact that there have been additions to the Staff, and it was felt desirable that the work of new members should be included, and in part to the increasing difficulty of finding leisure to compile and issue records of this magnitude. Indeed, on the present occasion, it was found necessary to split the Report into two volumes. For a time also we were sorely hampered by the effects of the disastrous fire to which allusion was made in our Third Report, and had to start building up once more, without thoughts of output or future publications. Our difficulties were, however, greatly minimised by Mr. Wellcome's speedy and ample aid, and by the help afforded us by the Director of Education and his Staff.

Sad to say, no sooner was some progress again being made than we lost by death our very capable and energetic laboratory assistant, Mr. E. O. Inglis. He had only been six months in Khartoum, but had already proved himself a man of the right stamp, and his loss was greatly deplored. His place was difficult to fill, but eventually we were fortunate enough to secure Mr. George Buchanan, who, having been trained under Mr. Richard Muir in the pathological laboratory of Edinburgh University, had gained much insight into laboratory methods, and, being an artist of no little skill and a good photographer, has been able to render admirable service. Shortly afterwards a second assistant, in the shape of Mr. A. Marshall, was appointed, as it was impossible for one man to cope with the ever-increasing routine work, museum work, oversight of experimental animals and equipment, and all the manifold duties which fall to the lot of a laboratory assistant, and which are much more onerous in the Tropics than in a temperate climate.

New Assistants

It was, however, evident that additions to the Scientific Staff were absolutely essential, and as a result of an earnest appeal, strongly backed by the Director of Education, two new appointments were made.

Dr. James Thompson, late research student at the Lister Institute, arrived early in 1910, to take up the duties of senior assistant chemist. In addition to routine work it was specially desired that Dr. Thompson should conduct pharmacological research work on the obscure drugs and poisons employed by the natives and on such allied subjects as seemed worthy of special consideration.

At a later period, through the kind offices of Sir William Leishman, F.R.S., the services of Captain W. B. Fry, R.A.M.C., were enlisted as Assistant Bacteriologist and Protozoologist. Unfortunately, Captain Fry had not been long with us, before, as a result of laboratory infection, he contracted Malta Fever and had eventually to return to England on sick leave. This has upset his research work on animal trypanosomiasis, which consequently does not figure so largely in this Report as would otherwise have been the case. Captain Fry's knowledge of some of the newer methods and up-to-date procedures has, however, been of great service.

The routine work continues to increase both in variety and magnitude. Since the date of the last Report nearly 1,800 examinations have been conducted in the bacteriological laboratory alone, comprising Wassermann tests, the preparation of bacterial vaccines, reporting on veterinary material, and on all the usual class of specimens which are sent in from hospitals and by practitioners. Certainly more use is now

Increase in the work of the Laboratories

made of such facilities as the laboratories afford, not only in the directions mentioned,

Water-supply

but in hygienic work, more especially of course for Khartoum. Thus, a prolonged investigation on the proposed water-supply for this city taxed severely the resources both of the chemical and the bacteriological departments and left but little spare time for research. As will be seen, however, this water enquiry, which turned out to be one of great interest and complexity, soon assumed the nature of a research which led, I believe, to the satisfactory solution of an important and difficult problem.

These are but a few examples of the purposes which the laboratories now serve. The scope of the work and details regarding it will be considered immediately, but here one would chronicle a new development, namely the advent of private workers who, at their own charges, have spent a considerable time in Khartoum carrying out research work in the laboratories and, for the sake of gaining experience in tropical work, kindly assisting the Staff in routine and other work. Thus, during the winter 1908–9, Dr. A. C. Stevenson of the University College Hospital, London, was one of these volunteers and he rendered good service by discovering coccidiosis in Sudanese goats,

Private Workers

an observation which enabled the veterinary officers to take measures for stamping out the disease amongst infected herds. Dr. Stevenson also investigated the hæmatozoa and intestinal protozoa of the common toad in Khartoum, and he contributes papers on both these subjects to this Report. He has also kindly supplied the drawings illustrating them. Dr. W. M. Aders, our other free-lance, was here at the same time as Dr. Stevenson. In addition to helping us with his zoological knowledge he studied the subject of hyper-parasitism, and contributes a paper on his researches into the Herpetomonas parasite which he found in *Aspongopus viduatus*, the melon bug of the Sudan.

Since these gentlemen left, applications have been received from an Italian Scientist, an American Professor and a medical missionary resident in Persia, for permission to study tropical medicine in the laboratories. Unfortunately, we have little spare room at our disposal, but in one of these cases arrangements have been made to grant the request, and Professor E. F. McCampbell of the Ohio State University, Columbus, will, I hope, be our guest and fellow-worker during the winter of 1911–2, both in the main laboratories and in the floating laboratory. The latter has been most usefully employed in the service of the Kala-azar Commission on the Blue Nile, and reports of the work done will be found included. The subject of transmission in this disease is a very difficult one, but Lieut. W. E. Marshall's careful observations go some way towards solving the problem.

Before proceeding briefly to outline the work accomplished in the different sections, one has to record the fact that, at Mr. Wellcome's request, the distinguishing word

"Tropical" added to title of the Laboratories

"Tropical" has been added to the title of the laboratories, and they will hereafter be known as the Wellcome Tropical Research Laboratories. As mentioned, the laboratory work has so greatly increased, and the number of our outside contributors is on this occasion so numerous, that it has been found advisable to split the Fourth Report into two volumes, the first dealing with medical, the latter with general science. At the time of writing, it is also hoped to bring out a Second Review Supplement more or less on the same lines as that issued as an appendix to our Third Report. We have received a good many letters testifying to the utility of that publication and expressing a hope that another volume would be forthcoming. Hence, both for our own sakes and for workers in the Sudan and

in other tropical countries, it seems advisable to make an effort to produce another compilation of this kind. Indeed, it is intended to extend its scope to the whole subject of tropical medicine so that it may be more generally useful. The difficulty, however, will be to keep it within reasonable limits as regards size. We will now consider the contents of Volumes A and B with such comments as may seem desirable.

Volume A represents the research and routine work done in the bacteriological section, but also, I am glad to say, includes a paper by Lieut.-Colonel H. B. Mathias, D.S.O., R.A.M.C., President of the Sleeping Sickness Commission, on the question of this disease in the Sudan. It is illustrated by maps showing fly areas and the distribution of the endemy in the Lado District, and represents the great amount of careful investigation carried out by the officers of the Egyptian Medical Corps who have been detailed for special service in the Southern Sudan. The laboratories are very much indebted to Lieut.-Colonel H. B. Mathias, not only for his valuable paper, but for his constant aid and support in his joint capacities of Principal Medical Officer of the Egyptian Army and Director-General of the Sudan Medical Department. I note that in his Annual Report for 1909 he writes as follows: "I would like to point out here what a valuable asset such a well-equipped Laboratory, with well-trained observers, is in Khartoum.

Contributors to Volume A

"Had such facilities not existed for research work, a large expenditure must necessarily have been incurred by the Government this autumn, in carrying out precautionary measures when a case that was clinically one of Asiatic cholera occurred, but which was proved not to be so by bacteriological examination.

"Moreover, by enabling a rapid and correct diagnosis to be arrived at in cases of certain diseases, such as kala-azar, malaria, diphtheria and enteric fever, etc., individuals derive the greatest benefit, as well as the community at large."

Naturally, such an expression of opinion from one so well qualified to judge is very gratifying to those responsible for the laboratory work. I quote it here as a powerful argument in favour of a statement which I propose to make later in this introduction (*vide page* 29).

Other valued contributions to Volume A are those by Captain L. Bousfield, R.A.M.C., who, though unfortunately no longer in the Sudan, has kindly given us an account of his experience in kala-azar and relapsing fever cases, and to whom the laboratories owe much for his kindness in presenting museum specimens and furnishing notes and information. The papers, by Captain Douglas Thomson, R.A.M.C., and Lieutenant W. E. Marshall, R.A.M.C., who formed the research members of the Kala-azar Commission, have already been mentioned.

In the spring of 1909, the laboratories were visited by Colonel Sir David and Lady Bruce, who were on their way to England from Uganda. Sir David Bruce kindly discussed with me the whole question of animal trypanosomiasis in the Sudan, and he and Lady Bruce were good enough to examine my preparations. Sir David laid stress on the confused nomenclature which existed, and outlined a scheme which he intended to follow out and which gave every promise of simplifying what is undoubtedly a difficult problem. At a later period our conference was resumed in England, and it was agreed to adopt Bruce's method when dealing with the trypanosome diseases of the Sudan.

Animal trypanosomiasis

In the meantime, Captain Fry had joined our Staff, and so it was arranged that he should be entrusted with the work. Lady Bruce most kindly undertook an extensive series of special measurements of our trypanosomes and prepared careful drawings of

them. Some of these have been incorporated in Plate I. which is most useful, as it had been found that the trypanosome plate in our Third Report had, through some misunderstanding, not been drawn correctly to scale, and hence gave a wrong impression of the size of the different parasites. As has been explained, Captain Fry's illness prevented him doing as much as could have been desired, but his preliminary work will be found detailed, and fresh light has been thrown on the subject by some observations of Mr. Buchanan, who, in our most common species of trypanosome, has discovered an endoglobular stage in the life history resembling that described by Chagas for *Endotrypanum cruzi*.[1] To him also are due the few details forthcoming as regards cultivation. The work has been hampered by the lack of strains. It is not difficult to get the camel and the common horse trypanosome, but it is not easy to secure strains of the cattle parasite (*T. nanum*) or of *T. vivax* (*cazalboui*) and other forms which may exist. As, however, Captain Archibald is proceeding to the Lado District with the floating laboratory in connection with sleeping sickness work, it is hoped to secure some of the less well-known strains which urgently require working out.

Work of the Director and his Staff

A glance at the contents of Volume A will show that Captain Archibald certainly cannot be accused of lack of diligence, and when it is remembered that the chief burden of the routine work has fallen on his capable shoulders, it will be apparent that his time has been fully occupied. Usefully so, also, one may say. He has discovered the existence of human botryomycosis in the Sudan, the presence of a special fever due to a specific microbe, the occurrence of acid-fast bacilli like *B. tuberculosis* in the lungs of camels, a form of cutaneous leishmaniosis not hitherto found in the Sudan, and, in addition, has undertaken a laborious piece of work on the bacteriological examination of water with a view to comparing conditions in the Sudan with those at which Clemesha and his assistants have worked so assiduously in India. Nor is this all, for during my absence on leave he had charge both of the laboratories and of the public health work for the town, and from the sanitary standpoint has tested the value of the Egyptian zeer as a water filter.

My own work has been very varied, and I could have wished that it had been more thorough, but one's time is so taken up with administrative and clerical work that it is hard to obtain what may be called consecutive leisure. Still, the increase in the Staff has relieved one from a good deal of the routine examinations and has made some research possible.

Work on fowl spirochætosis

The work on fowl spirochætosis, a puzzling problem, has been continued with some success in certain directions, more especially as regards the changes undergone by spirochætes ingested by the ticks which serve as vectors. The results confirm those obtained by Leishman in human tick fever, and indicate how important is the study of comparative pathology in the Tropics. Moreover, recent observations by special methods have, I believe, settled the question of the life-cycle of the parasites, both in the fowl and in the tick, and at the same time have demonstrated the important rôle played by the "infective granule" in this spirochætosis. These results may yet be found to have an important bearing on African Tick Fever and on other spirochætal infections of man.

An incomplete research on the spirochæte of Egyptian relapsing fever went some way to show that we were dealing with the newly-found *Sp. berbera* of Algeria and to indicate

[1] Chagas, C. (August, 1909), "Über eine neue Trypanosomiasis des Menschen." *Mem. Instit. Oswaldo Cruz.* T. 1. f. 2.

that the human spirochætosis of North Africa is due to this protozoon which, in all probability, is a specific entity.

It has seemed worth while to compile a paper on the fallacies and puzzles met with in blood examinations in the Tropics and elsewhere, and also again to deal with the broader aspects of sanitary work. A popular, rather than a scientific, paper gives some idea of the life of the sanitary inspector in Khartoum, and was originally written as an address to sanitary inspectors in England. Under Sanitary Notes the whole question of the water-supply of Khartoum forms an interesting and I hope instructive paper, and shows how necessary it is for all departments to work harmoniously together for the welfare of the public health. Of the papers on Sudan skin lesions, the first is the most important, for the peculiar non-ulcerating form of so-called "Oriental Sore" had hitherto escaped attention and formed a good subject of study, though here again the work was curtailed for lack of time and material. As an introduction to Captain Archibald's paper, one has inserted some notes on fevers in the Sudan, more for the purpose of drawing attention to how little we know about them than giving any full description of them, which, indeed, with our present knowledge is impossible. Diphtheria, however, receives a special article to itself, as I believe a curious coccal form of the diphtheria bacillus has been met with in the Sudan. I have ventured to present a paper entitled Veterinary Notes, for a great deal of our work here is concerned with diseases of animals, and, in the absence of any expert on veterinary laboratory work, these few lines may help the Sudan veterinary officers and, along with the Review Supplement, be some return for the many kindnesses they have shown the laboratories. More especially would one mention in this connection Captain F. U. Carr, the Director of the Veterinary Department, and Captain G. P. Knott, A.V.C. The latter takes a very keen interest in the work and rarely returns from tours of inspection without submitting material for examination and presenting specimens for exhibition. One may record the fact that Theiler's Anaplasmosis of South African cattle has been found in donkeys in the Sudan, and is here described.

Additional contributions

The other papers scarcely call for notice except, perhaps, that on the discovery of X bodies in human blood. For several reasons the work on this curious and rare condition could not be carried to a finish, and indeed this is one of the chief difficulties of research in a country like the Sudan. We need days double their present length, we require more facilities for seeing cases, though, so far as the Military Hospital in Khartoum goes, these are most generously afforded, we want more help and more money and more opportunities for instructing native medical officers so that they can send in material for examination in a proper manner and thus, in certain blood collections, minimise the risks of bacterial impurity. Still, things are improving, and the recent issue of regulations by the Sudan Medical Department, with full instructions as to the preparation and forwarding of material for laboratory examination, marks an important step in advance.

Need for help in collection of Material

Lastly, as regards Volume A, one must mention Dr. E. V. Crispin, Major S. O'Grady, R.A.M.C., and Captain Douglas Thomson, R.A.M.C., as kind contributors of clinical notes; while Major F. F. Carroll, Major H. Ensor, D.S.O., and Captains W. Byam, A. G. Cummins, C. M. Drew, R. J. C. Thompson and W. H. S. Burney, R.A.M.C. (attached to the Egyptian Army), together with Mulazim Awal Iskander Eff. Sarkis, E.M.C., have been at pains to furnish us with records of cases. So also,

on the Civil side, have Drs. V. S. Hodson, H. C. Squires, O. Atkey, G. R. Footner, S. Saigh and N. Mãloûf of the Sudan Medical Department. To Dr. Waterfield we are again indebted for museum specimens. Major W. Erskine, R.A.M.C., while in Khartoum in charge of British troops was most diligent in his attendance at the laboratories, where he did much work on Widal reactions, testing the permanency of the effect of anti-typhoid inoculation on the men of the Coldstream Guards. Major J. C. Jameson, Captain Harvey and Captain O'Farrell have since that time also spent much of their leisure at the benches. The last named has, moreover, very kindly aided in the compilation of the Review Supplement.

Volume B. Turning now to the second volume, it will be found that its contents range over a considerable variety of subjects, Chemistry, Entomology, Ornithology, Helminthology, certain Arachnida of the Sudan, Protozoology, Geology, Anthropology, Sociology and Sanitary Engineering all claiming attention.

Contributors to Volume B

Again we are indebted to extra-mural workers. Mr. A. L. Butler, at my request, kindly furnished a useful practical paper on the grain-eating birds of the Sudan, which, fortunately, thanks to Mr. Wellcome, it has been possible to have illustrated in a manner worthy of the text. Dr. Leiper,[1] for the second time, favours us with an account of Sudanese parasitic worms, and Professor Werner, of Vienna, with a most interesting paper on scorpions and annulated spiders which, with its fine illustrations, cannot fail to be of value. Sir Thomas R. Fraser, of Edinburgh, has very kindly given us a paper on the venom of the Sudan "Spitting-Snake," and Mr. Henry Curtis has written an account of the "Spitting-Snakes" of South Africa, which is illustrated by drawings made by Lady Dorothy Stanley. Mr. Dunn contributes an interesting account of ancient gold mining in the Sudan and Mr. F. V. Theobald notes on two new mosquitoes.

Dr. Aders' paper has been mentioned, and Captain Anderson, whose monograph on medical customs in Kordofan was one of the features of our last Report, has again put pen to paper on our behalf, and contributes a most interesting paper on the Nyam-nyam and Gour people inhabiting the Eastern Bahr-El-Ghazal. The chief anthropological interest is this year supplied by Dr. C. G. Seligmann's paper. He and Mrs. Seligmann, in 1908-9, were given the laboratory steamer for their journey south, and in return Dr. Seligmann has kindly become a contributor, dealing with subjects on which he is a well-known expert.

Finally, Messrs. W. H. McLean and G. E. Hunt, of the Gordon College, who have aided me so greatly in the public health work of the city, have compiled a practical paper on such aspects of tropical sanitary engineering as are likely to appeal to those who have to cater for the sanitary needs of towns in tropical countries, and which should be most useful to Medical Officers of Health, Sanitary Inspectors, Municipal Engineers, Boards of Health and other local Sanitary authorities, at least such as have to work in hot, dry and dusty countries like the Sudan.

Non-medical Members of the Staff

Turning now to the work of non-medical members of the Staff, one finds that Mr. King has been most assiduous in his undertakings. As, at the beginning of his report, he gives an outline of what he has accomplished, where he has been and what he intends to do, I need not enter into details, but content myself with saying that Mr. King is a very valuable asset to the Sudan, and that every facility should be given him to make the most of his keen interest in, and sound knowledge of, his subject.

I need merely refer to his researches on the bionomics of Tabanidæ, which are now

[1] Unfortunately the paper by Dr. Leiper was not received in time for publication.

quoted in all works on this subject, to the Sudan Plant Diseases Ordinance, which owes its existence to his efforts, and to his notes on mosquitoes and mosquito-devouring fish, to indicate what an essential factor this economic entomological work is in the well-being and for the future of the Sudan. A most gratifying feature is the help rendered Mr. King by well-nigh everyone, from Governors of Provinces downwards.

One leaves the most important items to the end as a rule, working, as it were, towards a climax, and hence it is only fitting that this place of honour, so far as Volume B is concerned, should be devoted to a brief consideration of the work of the chemical laboratory. Although Dr. Beam has certainly not received that outside aid which should have been fully and cordially extended to him, yet, I think, there are now few in the country who will deny how great a bearing his laborious and painstaking investigations into Sudan products and soils have on the development of the country. He has undoubtedly been hampered and hindered in his work by those who, without proper scientific training, could not appreciate the necessity for scientific methods of collection and procedure, and, I fear, resented advice on these essential points. I believe, however, that the worst is past and that those responsible for the development of commercial products are now likely to co-operate and to recognise how absolutely essential it is to abandon slipshod measures and to proceed on sound scientific lines. This is specially necessary in the case of soil investigations which now bulk so largely. The locality which is being chiefly studied is the Gezira, that vast area lying between the Blue and White Niles, which, if properly handled, may yet bear great crops of wheat and of cotton. There is a scheme afoot to place it under perennial irrigation, and, as the cost of the irrigation work will be very great, it is essential that a sound knowledge of the character of the prevailing types of soil be secured before any reliable opinion can be expressed as to the success or otherwise of such an undertaking. As Dr. Beam says, "attention has been called to the paramount importance of a study of the subsoil as well as of surface conditions, since the question of drainage, resistance to drought and root penetration will depend essentially upon the nature of the substrata. Mechanical analyses of these soils and their subsoils (*i.e.* the determinations of the proportions of gravel, sand, silt and clay) are therefore being made in all cases, and determinations of the proportion of the essential plant constituents, potash, phosphoric acid and nitrogen as well. These results, taken in conjunction with the observations made in the field, should yield sufficient evidence to enable one to form at least a reasonably accurate judgment as to the results which may be expected from irrigation if the cultivation is carried out on suitable lines."

Work of the Chemical Laboratory

Analyses of Soils and Subsoils

Soil samples are also being obtained from Dongola and other districts, so that a good general idea may be obtained of the classes of soils met with in areas which are, or are likely to be, cultivated; while the prospect of increasing the yield of certain crops by scientific treatment is shown in a most interesting paper on "Gypsum as a Fertiliser for Sudan Soils." Moreover, after a prolonged and careful study of the question of soil examination, Dr. Beam has devised new, rapid and special methods, which not only greatly facilitate his own work, but appear to place the physical examination of arid soils on a sounder basis than it has hitherto occupied. These are fully described. The gum work, which for reasons explained by Dr. Beam, had perforce been abandoned, is shortly to be resumed with, it is thought, better chance of successful results. Mr. Edie again contributes a paper on the subject, having kindly continued and

extended his observations in England. Of special interest are his conclusions, based on Dr. Beam's suggestions, as regards the rôle of ants in gum production, an interesting analogy, possibly, between the transmission of diseases affecting animal and vegetable life respectively.

The Poisonous Ushar plant

Dr. Thompson, besides assisting in the work on soils, water, poisons, commercial products and the various analyses which are always being conducted, took up as a line of research the pharmacology of *Calotropis procera,* the Ushar plant, with a view to isolating the toxic principle or principles which, as previously explained, may occasion poisoning by merissa, the national Sudanese drink, a kind of beer brewed from dura—(millet). His results are detailed in his paper. In this connection one would gratefully acknowledge the kind help received from Dr. Power and Mr. C. J. S. Thompson of the Wellcome Chemical Research Laboratories in London, who furnished records of the literature on the subject to which we could not otherwise have easily gained access.

Dr. Beam, himself, has succeeded in finding a colour test for the detection of hashish, a most useful discovery the value of which has been confirmed by Professor Schmidt and Mr. Lucas in Cairo, and has been concerned in all kinds of analytical and advisory work, the outcome of questions brought before the Central Economic Board.

Mr. Goodson, who has been elected a Fellow of the Institute of Chemistry, has rendered valuable assistance and has also had charge of the meteorological work for Khartoum, a duty which I am informed he discharges in a most satisfactory manner, but which naturally trespasses somewhat on time which might well be devoted to chemical investigations when there is so much to be done.

The *S. W.* "*Culex*"

The laboratory steamer has been mentioned. It was named the "*Culex,*" and has been well-nigh as ubiquitous as its namesake. After conveying the Floating Laboratory up the Blue Nile, it took Dr. and Mrs. Seligmann up and down the White Nile. Then Colonel Mathias and I travelled by it to the Bahr-El-Ghazal where a trypanosome strain was secured and where experiments were made with a Dinka tsetsefuge in the shape of *Vitis producta.* So far the outlook in this direction is not promising, but Mr. King, who is now working for the Sleeping Sickness Commission, is to repeat and amplify the observations. Cattle diseases were also studied and posts on the Nile visited and reported upon at the instance of the Central Sanitary Board. The *S. W.* "*Culex,*" then, is a most useful auxiliary, and greatly enhances the value of the laboratory barge, making the latter independent of the ordinary Nile steamers.

The laboratories' museum has been housed in a new and larger room kindly granted for the purpose by Mr. Currie and, under the fostering care of Mr. Buchanan, has considerably developed, though one still regrets the serious losses caused by fire. It is, however, in a better state generally than has ever been the case before. Dr. Waterfield has kindly sent some very interesting material from Port Sudan, and a circular which was sent out has produced good results on the whole.

The Library

The library increases slowly, for our funds are limited, but our exchange list has attained formidable dimensions and the work of checking, acknowledging and cataloguing reprints and other publications is no light matter considering we do not possess a secretary or librarian.

One again takes this opportunity of thanking all those, and they are now to be found in most parts of the world, who have been good enough to send us reprints of



G. Buchanan

Fig. 3.—Sectional View of the Pathological Museum



G. Buchanan

Fig. 4.—Sectional View of the Pathological Museum

their work. Thanks are also due to the Editors of the *British Medical Journal, Lancet, Journal of Tropical Medicine and Hygiene, Journal of Hygiene, Journal of the Royal Army Medical Corps* and *Journal of Comparative Pathology and Therapeutics* for kind permission to reproduce papers in this Report.

So many Sudan officials have given us kindly aid that I cannot attempt to mention all of them here. I have already referred to the medical officers who have helped us, and Dr. Beam and Mr. King make their own acknowledgments. I would, however, specially like to thank Captain A. L. Hadow, because, though not a medical man, he has taken a great deal of trouble to secure specimens of poisonous snakes, of venom and other objects of interest, while the note on venom by Sir Thomas R. Fraser, and an account of "Spitting-Snakes" by Mr. Henry Curtis have already been mentioned. My thanks are also due to El Kaimakam C. V. N. Percival Bey. I may state here that Major Lyle Cummins, R.A.M.C., late of the Egyptian Medical Service, has kindly acted as the Editor in England of this Report, an arrangement made by Mr. Wellcome, and one which has helped me very greatly. Many of the illustrations have again been entrusted to Mr. Richard Muir whose skill requires no encomiums from me.

Distinguished Visitors

The laboratories have been visited by a number of persons interested in their work. I have mentioned Sir David and Lady Bruce. Captain F. P. Mackie, I.M.S., followed them, also on his way from Uganda. Monsieur A. Solvay, of Brussels, came and saw and, as on a previous occasion, most kindly gave us a generous donation which enabled us to purchase a special microscope for the chemical department and to secure some books of which we had need. I only wish more visitors to Khartoum would follow M. Solvay's example. Perhaps they would if they understood the laboratories stand, in some degree, for health, and health means comfort and prosperity and a good many other things which make life worth living. It is Emerson who says, "The first Wealth is Health," a motto which might suitably be inscribed upon our walls and exhibited at intervals to those who control our finances! Lord Kitchener also inspected us and, with his Indian experience, showed himself a keen, though happily a friendly, critic. One has also to record Mr. Wellcome's return to the Sudan. Needless to say we were pleased to see him, and he went fully into all matters pertaining to the laboratories, and, as ever, showed much interest in their development. He had recently been in Panama, studying the Sanitary Department work, and it is remarkable how many of the problems in the Sudan resemble those in the Canal Zone though, of course, on an infinitely smaller scale. There are, however, the same difficulties and disappointments in both places, and one derives a kind of vicarious comfort from such a reflection. At the same time we still retain the support of H.E. the Governor-General, while to Mr. Currie, to whose far-seeing grasp of affairs the establishment of the laboratories was primarily due, we are more than indebted. Under a less sympathetic, a less influential, or, I may add, less determined chief I am quite certain that the development of the laboratories would more than once have received a serious check, not from any hostility towards them but from a lack of knowledge of their needs and a lack of appreciation of all they represent and of Emerson's sound dictum. In the past, one has always concluded an Introduction by considering the future, and, after eight years of experience, one has now a good idea of what is and will be actually required to keep these laboratories abreast of the times.

Requirements

In our Third Report I spoke of the necessity of a new building, in this I will deal

with the Scientific Staff as it should be. Here is what we want, what we have in part obtained, and what I trust will yet be forthcoming:—

Scientific Staff:
Director

Bureau of Microbiology:
Bacteriologist
Pathologist
Veterinary Pathologist
Protozoologist
Helminthologist

Division of Entomology:
Two Economic Entomologists, one especially concerned with medical entomology

Division of Botany:
Botanist
Mycologist

Bureau of Chemistry:
Research Chemist in Charge
Economic Chemist
Agricultural Chemist
Pharmacological Chemist, whose work would include toxicology

In time, no doubt, it would also be well to have an Assistant Director, a Hæmatologist to carry out serum tests such as Wassermann's reaction, other complement fixation and precipitant tests for blood, and such new developments in this important diagnostic branch of medicine and surgery as may arise. An Anthropologist would also pay his way, especially if his main work was in the direction of sociology.

These then, with the necessary assistants including an artist, with an adequate clerical staff, and more especially with a secretary who could also look after the library, would supply a force which might wage a most successful war with disease in man and animals and plants, which might make the most of such products as the Sudan yields and indicate how they might be improved, which might point out along what lines agriculture should develop, and aid those who will irrigate and till what is now a virgin soil. They will cost money but they will save money, and indirectly they will make money. Money, however, is after all not everything. "The first Wealth is Health," and given health all other things may be added unto us, even in a country like the Sudan with its manifold drawbacks and disadvantages. When I asked Sir David Bruce what he thought should be our annual contingent he straightway replied "ten thousand pounds." We are a very long way off ten thousand pounds, but one is not without hope for the future when one sees how everywhere Science leads the way. We have already grown from small beginnings to a respectable stature, are, I think, secure against extinction, and, so long as we proceed on practical lines which have a bearing on the development and progress of the country, should not fail to receive that support and consideration which the magnitude, scope and importance of the work that now falls to our share amply justifies.

"The first Wealth is Health"



FIG. 5.—MAP OF ANGLO-EGYPTIAN SUDAN

Sleeping Sickness in the Anglo-Egyptian Sudan

being a

NOTE ON THE ADMINISTRATIVE MEASURES THAT HAVE BEEN TAKEN IN THE SOUTHERN SUDAN TO DEAL WITH THE ADVANCE OF SLEEPING SICKNESS NORTHWARDS FROM UGANDA, THE LADO, BELGIAN CONGO AND FRENCH CONGO

BY

Colonel H. B. Mathias, D.S.O., R.A.M.C., P.M.O.E.A.

President, Sudan Sleeping Sickness Commission

Dr. Andrew Balfour has asked me to write a short note on the above subject for the Fourth Report of the Wellcome Tropical Research Laboratories and I gladly do so.

In the Third Report (1908), extracts from Captain Howard Ensor's report to the Sudan Sleeping Sickness Commission appeared, and in these extracts all that was then known of the distribution of tsetse flies, both *G. palpalis* and *G. morsitans*, in the Bahr-El-Ghazal was set forth, and the question as to whether sleeping sickness existed at that time in the Province discussed, without its being possible to give a definite answer one way or another. Certain recommendations were, however, made by this Officer, and, acting on these, and from the general information contained in his report, it was decided by the Sudan Government to despatch a British Medical Officer in the autumn of 1908 to the Bahr-El-Ghazal, and Captain R. G. Anderson, R.A.M.C., attached to the Egyptian Army, was selected.

British Medical Officer detailed for "Sleeping Sickness" Duty

He was ordered to travel over, and investigate the conditions existing in, the southern portions of the Bahr-El-Ghazal (its southern frontier and immediately adjacent states) which have a more direct bearing on the gradual invasion of sleeping sickness from the south.

Leaving Khartoum in November, 1908, he proceeded to Shambe on the White Nile, thence south by way of M'volo and Meridi to the River Sueh, carrying out the routine examination of the inhabitants by the way and afterwards returning and traversing the northern frontier of the Lado. He paid special attention to the upper portion of the River Yei, and the villages Amadi, Dego and Dai, which were suspected centres of sleeping sickness, and then crossed to the southern limits of Mongalla Province on the Nile. With regard to the information obtained by him, it will be sufficient to mention here that not a single case of sleeping sickness could be found, and that after five months of arduous travel, as the rains were starting, making further progress in the southern Bahr-El-Ghazal almost impossible, he was ordered to return to Headquarters and write his report.

Natural barriers against Sleeping Sickness

Captain Anderson brought to notice among other things the important fact that a barrier exists to the east of the Bahr-El-Ghazal against the inroad of sleeping sickness, consisting of a large area devoid of permanent open water of any sort, which stretches from the River Yei to the Nile. Also in Mongalla Province, between Mongalla station and Gebel Lafone (some 60 or 70 miles) there is an extensive plain, very thinly populated, devoid of permanent open water and free from tsetse flies, and farther eastward still, towards Abyssinia, the country is reported to be desolate and uninhabited. On reference to Map (Fig. 7) it will be observed, as pointed out by Captain Anderson, that

the Yei River route to the Bahr-El-Ghazal from the Lado, and the Nile route from Uganda, can be controlled by Inspection Posts at M'volo and Mongalla respectively, and measures were at once taken to have Medical Officers stationed at each of these places for that purpose.

Beyond rumours (alluded to in Captain Anderson's report) of sleeping sickness in the Lado, and information obtained through the courtesy of Dr. Errara, of the Belgian Government Service, nothing further was heard of sleeping sickness in the southern Sudan until October, 1909, when the Senior Medical Officer, Bahr-El-Ghazal, wired, saying that the Medical Officer at Ragaa had reported that there were some suspected cases at that station, which is near the western boundary of the above-named Province. He asked permission to proceed and investigate, and for the services of the British Medical Officer engaged on sleeping sickness work. Captain C. M. Drew, R.A.M.C., attached to the Egyptain Army, was despatched to the western Bahr-El-Ghazal in December, 1909, and Captain (now Major) Ensor, D.S.O., R.A.M.C., who was going to the Bahr-El-Ghazal as S.M.O. in relief of Major Carroll, accompanied him.

Cases reported from the Western Bahr-El-Ghazal

Both Officers were appointed members of the Sudan Sleeping Sickness Commission. Captain Drew was ordered to visit as much as possible of the western Bahr-El-Ghazal and to enquire into the prevalence of sleeping sickness, to map out fly areas, and to devise measures for the prevention of a possible invasion of the disease from the infected districts of the French Congo.

Major Carroll and Captain Ensor met at Wau and, in consultation, sent in recommendations as to the precautions that were necessary with regard to traders, porters, and other persons crossing the frontier, which were embodied in an Ordinance whereby all porters are stopped at the frontier posts and turned back, animal transport being used to convey the goods subsequently.

Quarantine Ordinance

By reference to Figs. 6 and 7 it will be observed that Isolation Camps have been established at Ragaa and Dem Zubeir, and Inspection Posts on the main trade routes from the French Congo to the Sudan, viz., at Dem Zubeir, Sabun and Said Buldas respectively.

This scheme of quarantine has been found to deal adequately with the supervision of all classes except refugees. All the merchants and the majority of the natives use the roads, but the refugees make their way through the forests and avoid the posts. The only way of detecting and dealing with them is to insist on the Sheikhs and Sultans bringing all new-comers to their villages to the Government Posts for medical examination, after having been thoroughly warned of the danger of harbouring such people. This arrangement has been carried out and has been the means of detecting one case of sleeping sickness.

In addition to the above methods, clearing the fords of *palpalis*-haunted khors on the main road from Wau to Kafia Kingi (roughly 400 miles) was carried out, and extensive clearing done at the Quarantine Camps at Ragaa and Dem Zubeir, and the Inspection Posts at Biri and Said Buldas. The banks of the river at Ragaa were cleared for about three miles, and, 300 yards back, similar extensive clearing was carried out at Said Buldas —these clearing operations will be carried out twice a year, in future, by local arrangement.

Clearing of river banks

Discovery of Sleeping Sickness Cases in the Western Bahr-El-Ghazal

Only two cases of sleeping sickness were found by Captain Drew at Ragaa, although it is thought likely that a few cases had already terminated fatally before he got there. They were all in natives who had come from the French Congo, the two positive cases being found among natives who had been detained in the Quarantine Camp by order of Major Carroll.



From a map compiled at the Survey Office, Khartoum

FIG. 6.—MAP OF NORTHERN BAHR-EL-GHAZAL SHOWING TSETSE FLY AREAS

Of Natives living entirely in the Bahr-El-Ghazal, Captain Drew examined 1400; of this number, 271 had enlarged cervical glands attributable to one cause or another.

The routine method of examination consisted of gland palpation, examination of fresh and stained blood films, and gland puncture.

Examination of Natives

When no obvious cause of gland enlargement was observed, an examination of the fresh blood was made, altogether 130 blood examinations being carried out; in no case were trypanosomes found or auto-agglutination noted.

Seventy-three persons *coming to the Bahr-El-Ghazal from the French Congo* and detained in the Quarantine Camps were also examined. Fifty were merchants and their servants, and twenty-three were natives desiring to settle in the Bahr-El-Ghazal.

Blood examinations were made in each case and all found negative, except two of the latter in which auto-agglutination was noted—one, an Arab, was obviously in the last stage of sleeping sickness, the other an apparently healthy man with the exception of some enlargement of the glands, and mental dulness. Trypanosomes were, however, found in the gland juice.

By the terms of an agreement with the Belgian Government, the Lado was taken over by the Sudan Government in June, 1910, and a Commission, of which Captain Colin Mackenzie, D.S.O., R.A.M.C., attached to the Egyptian Army, was a member, left Khartoum on April 14 for this purpose.

It was known that sleeping sickness existed in the Lado, and Captain Mackenzie was ordered, as far as time and circumstances permitted, to ascertain to what extent the disease was prevalent in the Yei Valley and other parts of the country. He was met on arrival by El Yousbashi Yusef Effendi Dervish, Medical Corps, E.A., who had been stationed at Mongalla for some time previously, and who had sent valuable information to Headquarters, which he had collected from various sources, of the amount of the disease in the Lado. This Medical Officer was sent ahead by Captain Mackenzie to Yei on May 16 to meet Dr. Errara, the Belgian Medical Officer of the Lado, and ascertain from him particulars of the various cases already known at and around the post. These cases were then to be brought before Captain Mackenzie on the arrival of the Commission at Yei in order that he might satisfy himself as to the diagnosis, before the occupation of Yei and Kagelu Posts came up for decision.

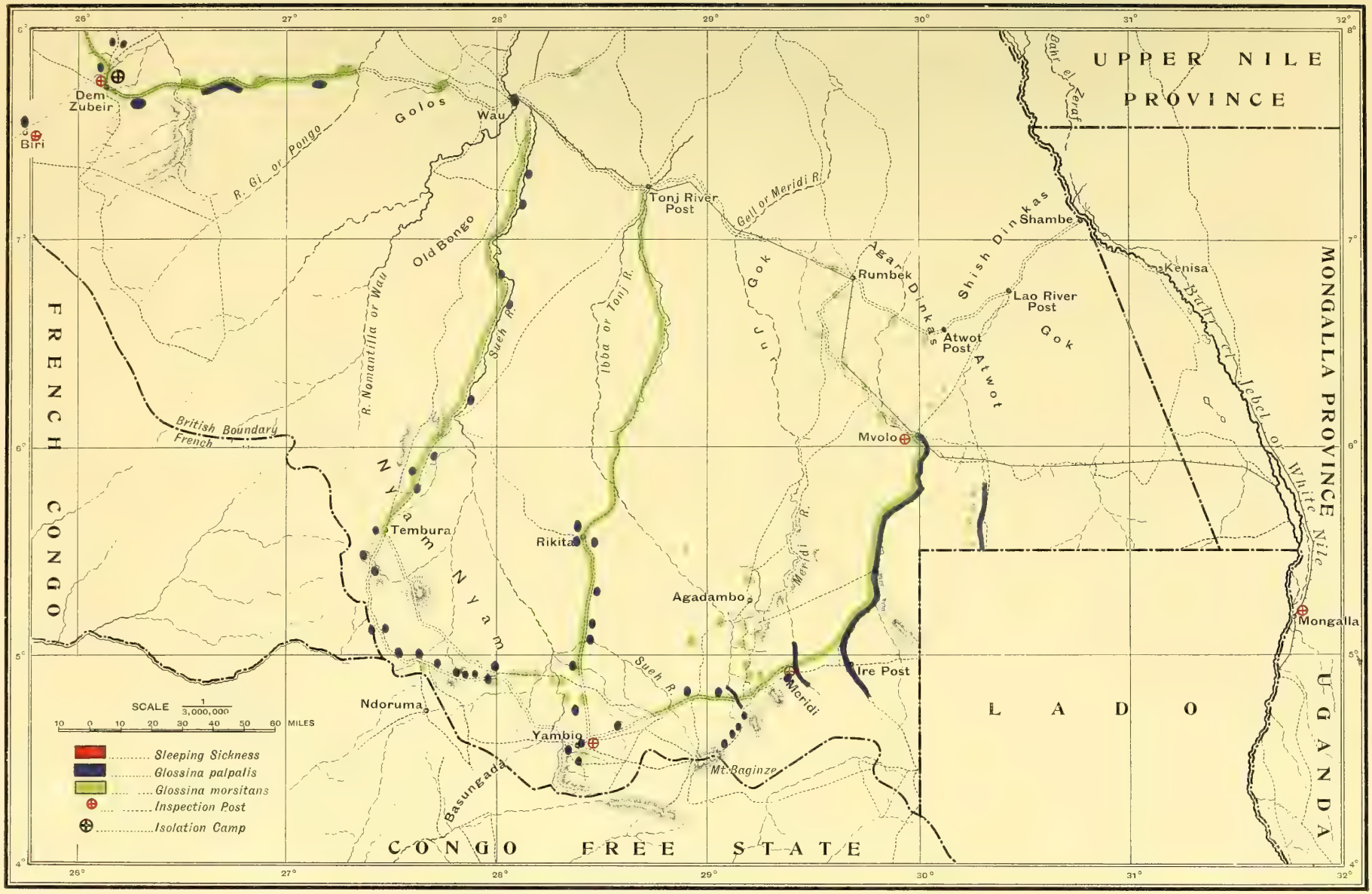
Sleeping Sickness in the Lado

Captain Mackenzie met Dr. Errara personally, later, and was informed by him that sleeping sickness had existed in the Lado for over two years and that he had reported the matter to his Government.

On June 8, at Yei, Captain Mackenzie found Dervish Effendi had collected fifteen cases of sleeping sickness, in eight of which he found trypanosomes, the remainder had been treated with atoxyl. Most of these people came from Wata and Lajuba, and eight of them were women or children. The women do most of the water-carrying, and probably were infected when drawing water from the river: "All seemed to take the greatest delight in having their cervical glands punctured, even the children showing no signs of fear!"

Search for western and northern limit of the disease

Dr. Errara also stated that, according to native reports, sleeping sickness exists far north in the Yei Valley, and, with a view of ascertaining the western and northern limits of the disease and of *G. palpalis*, Captain Mackenzie, after leaving Yei, proceeded to Libogo on the frontier, thence along the Congo watershed until the head waters of the River Tor were reached. He followed down the course of this river to Fudl-il-Mula, near Kapei on the River Yei, and next proceeded northwards to Waringua, Beringis village near Wandi, which was found infected. From Waringua, he next marched east,



From a map compiled at the Survey Office, Khartoum

FIG. 7.—MAP OF SOUTHERN BAHR-EL-GHAZAL SHOWING TSETSE FLY AREAS

crossing the Yei at Luri Rapids and thence north down the east bank to Lorella, and from Lorella, again east to Bundukia. This was one of the places where the natives reported sleeping sickness existed, but none could be discovered, neither was any found in the villages between here and Lado. *G. morsitans* was very numerous in places, but no *G. palpalis* was found farther north than Wandi.

As a result of his investigations Captain Mackenzie concludes:—

1. That a line drawn from Loka to Bangali and thence to Libogo, from there along the watershed to the source of the Tor, then down the Tor to just north of Beringi and thence to Loka, would enclose the area which is at present infected with sleeping sickness (Fig. 8).

The infected area

2. That there are probably a few isolated cases outside this area occurring in men who have been working and become infected in Yei.

3. That *G. palpalis* is to be found near water in most of the area above mentioned.

4. That *G. palpalis* also extends all along the River Tor and its tributaries, and also northwards along the River Yei. (Fig. 9).

In the meantime, another British Medical Officer was engaged to carry on sleeping sickness work, and, on the return of Captain Mackenzie, this Officer, Captain R. J. C. Thompson, R.A.M.C., went to the Lado as S.M.O., and was accompanied by two Egyptian Medical Officers to assist him. Owing to it being the rainy season, he has found that clearing is difficult, but a great deal has already been accomplished around Yei on both banks of the river, and *G. palpalis* is now reported as rarely seen.

The sleeping sickness cases are being marked down, and will shortly be collected in a camp in a fly-free area.

Regulations have been drawn up with regard to the importation of porters, and communication with the Bahr-El-Ghazal prohibited, and it is hoped, in a relatively short period, to have final arrangements for dealing with the disease in the Lado, complete.

In conclusion, I recapitulate the measures that have been taken as a result of recommendations advanced, and the information obtained by the Medical Officers of the Egyptian Army on behalf of the Sudan Sleeping Sickness Commission.

Summary of precautionary measures

1. An Inspection Post has been established on the Nile at Mongalla.
2. *Lado.*

(*a*) Mapping of the country showing fly areas, and areas proved to be infected with sleeping sickness.

(*b*) Notifying all cases, in order that they may be moved to a sleeping sickness camp as soon as possible.

(*c*) Clearing fly areas round Yei and Kagelu posts.

(*d*) Enforcing of regulations regarding traders, porters, etc.

3. *Bahr-El-Ghazal. Eastern Boundary.*

(*a*) Establishing an Inspection Post at M'volo, which latter station has been moved to a more healthy site in a fly-free area.

(*b*) Medical Officers at Meridi and Yambio warned to examine all natives arriving from the Lado, or Congo Free State.

4. *Bahr-El-Ghazal. Western Boundary.*

(*a*) Quarantine Stations and Inspection Posts established on the main trade routes.

(*b*) A vast amount of clearing carried out.

(*c*) Special regulations issued dealing with traders, porters and refugees from the French Congo.



FIG. 8.—MAP OF LADO SHOWING TSETSE FLY AREAS AND PROBABLE LIMIT OF SLEEPING SICKNESS

From a map compiled at the Survey Office, Khartoum

Difficulties on the southern frontier

In the south there is a long line of frontier where the Bahr-El-Ghazal adjoins the Congo Free State, the boundary line being the Nile and Congo River watersheds, where, owing to the physical conditions obtaining, any methods of prevention would appear next to impossible. The country is densely wooded, there are no main trade routes, merely small native paths through the jungle; the rains last for nine months of the year (March to November) and are the chief hindrance to inter-communication. Owing to the flooding of swamps and roads and the rapid growth of tropical vegetation which they cause, any inter-tribal communication taking place during the dry season is prevented. This district, moreover, is reported to be now thinly populated, so that short of an emigration northwards of a whole tribe *en masse* to escape from sleeping sickness in the Congo Free State (as suggested by Captain Ensor, in his 1907-8 report) the danger on this boundary does not appear to be an imminent one. It is hoped, however, should the services of Captain Drew be available later, to send him along this boundary in order to collect more definite information as to the present condition of affairs.

Additional Note

Since writing the above I have made a tour of inspection in the Lado accompanied by Captain R. G. Archibald, R.A.M.C., of the Wellcome Tropical Research Laboratories, Khartoum, and visited the Sleeping Sickness area.

Clearing measures adopted

I found an immense amount of work had been accomplished by Captain R. J. C. Thompson, R.A.M.C., and the two Egyptian Medical Officers, Mulazimin Awal Nesib Eff Haddad and Nesib Eff Bas, who are assisting him.

The majority of the khors between Loka and Yei, where they cross the Automobile Road, have been rendered fly-free.

The banks of the River Yei, for a distance of over three miles in the vicinity of Yei itself, have been thoroughly cleared of all vegetation. (Fig. 10.) During my stay of five days in Yei not a single fly could be found, unless an excursion was made on to the uncleared river bank, where there were plenty of *palpalis* in evidence. In addition to this, much clearing of vegetation, and drainage, have been carried out in and around Yei itself.

Kagelu Government Farm, some six miles west of Yei on the Automobile Road, has also been rendered fly-free.

Many of the villages in the sleeping sickness area have been dealt with, tools have been lent to the various Sultans and Sheikhs, and they have been encouraged to clear all trees and undergrowth from the neighbourhood of watering places.

At Yei, Captain Thompson has built an Isolation Camp and a good hospital for sleeping sickness cases (Fig. 11). There were 40 of these in camp undergoing treatment at the time of my visit.

Treatment.—This has consisted in intravenous injections of a special preparation of metallic antimony, which was kindly given us for experimental work by Mr. H. G. Plimmer, F.R.S. In addition, a certain number of cases were being treated with atoxyl, and others with "606."

Number of cases under surveillance

Captain Thompson is of opinion that he has got nearly half of the total number of cases in the sleeping sickness area of the Yei Valley now in camp. I am, however, doubtful on this point, and anticipate it will be found that the number of cases in this area will prove eventually to be nearly double this number. From the evidence of the natives themselves many of the smaller villages have already been decimated by the disease, with which the people are well acquainted, and which they appear to be able to recognise in the early stages.



R. G. Archibald

FIG. 9.—TYPICAL HAUNT OF *Glossina palpalis* ON THE YEI RIVER
There is a patch of sand at the water's edge



R. G. Archibald

FIG. 10.—CLEARING OPERATIONS ON THE YEI RIVER

It seems to me to be reasonably possible for the disease to be kept under control in this part of the Lado, provided that fresh infection is not introduced by means of porters coming from Uganda and the Congo. They are now being carefully examined at Yei, and the Sudan Government is being advised as to the framing of regulations for dealing with this danger.

Distribution of Fly

With regard to the distribution of tsetse fly in the Lado, I met Captain Drew, R.A.M.C., and Mr. King, Government Entomologist, in Yei. They had followed down the course of the River Yei from M'volo, in the Bahr-El-Ghazal (*vide* Map, Fig. 7), and reported that *palpalis* was present, both on the river and adjacent khors, throughout the entire route.

El Bimbashi Stigand, the Inspector at Rejaf, reported he had found *morsitans* and *palpalis* in khors north of the Automobile Road between Ganzi and Wande, and also showed us flies of both species collected from around Kajo-Kaji south of Rejaf. This fly area apparently extends into the hills in the neighbourhood and was formerly believed to be fly-free. Further reports having also been received of the spread of Sleeping Sickness northwards on the right bank of the Nile in Uganda territory, Mr. King has now gone to Kajo-Kaji to investigate into the northerly limit of *palpalis* on the Nile bank in the Lado.

Conclusion

Personally I am of the opinion that where suitable conditions of water and shade exist, there *palpalis*, *morsitans*, or both, will be found, and that the tsetse fly has a much wider area of distribution in the Lado than was at first suspected.



R. G. Archibald

Fig. 11.—Sleeping Sickness Camp, Yei

These types possess readily recognisable morphological characteristics which, at any rate, enable one to differentiate positively between the four.

It will be very apparent how desirable a system of general distinction is, when the extent of the Anglo-Egyptian Sudan is considered, and when it is remembered that, owing to natural conditions and geographical necessities, both animal and human life has a tendency to clump into diffuse and somewhat widely separated colonies or tribes. This occasions not only the discovery of apparently isolated types of the disease, but undoubtedly alters and influences the virulence and characteristics of trypanosomes which are of the same stock. Unless some system be carried out, terms such as the "Trypanosomiasis of Mules," "Trypanosomiasis of the Shilluk Cattle" and "The Disease of the Camels of Erkowit," come into existence, and, as they give no clue as to what the pathogenic agent is, endless confusion is caused thereby.

Necessity for a systematic nomenclature

While it is admitted that morphological distinctions alone are not ideal or completely adequate, yet they are of great practical utility in advancing towards intelligent classification, rendering further separation an easy matter, thereby saving us from the fallacies of identifying trypanosomes by their local hosts. The advance of the study of these diseases has been to show that the communicability of the infection is less limited than was at one time supposed, and that the restriction of a certain type of trypanosome to one class of animal is due as largely to the existence of a suitable transmitting agent as to the inherent refractory nature of the other animals.

We may at this point take a concise review of our four types.

Type 1. *T. brucei* or *pecaudi*, found on the Sobat and White Nile and in the Bahr-El-Ghazal; this trypanosome has been carefully investigated and reported on by Dr. Balfour in the Second and Third Reports, and the conclusion arrived at was that it most closely resembled what has been called the *T. pecaudi* of Senegal. Colonel Sir David Bruce, F.R.S., has, however, shown that, morphologically, it almost exactly resembles what he describes as *T. brucei*, a point more definitely considered in the second portion of this paper. Moreover, a comparison of the animal reactions shows few discrepancies. It is not intended here to discuss the position of *T. pecaudi* as to its specificity or otherwise, but it is obvious that it were better to include a doubtful trypanosome under the heading of a large and well-recognised class than to place it with a smaller and less old established genus, should the reasons for placing it with one or the other be in any way of equal weight.

Review of the Trypanosome types

It has been suggested that a reason for regarding Type 1 as *T. pecaudi* is, that while horses, mules and donkeys are particularly affected on the White Nile and in the Bahr-El-Ghazal, we have in these same districts herds of cattle belonging to the natives which are, at any rate, so far unaffected that the natives can maintain them and breed them in these regions, circumstances in some ways analogous with the occurrence of "La Baleri," whose pathogenic agent has been called *T. pecaudi*.

A brief consideration of a few points will show how fallacious a hasty suggestion of this kind may be; firstly, *T. brucei* produces a more chronic disease in bovidæ than in equidæ; secondly, cattle are not entirely refractory to *T. pecaudi*; thirdly, horses, mules, etc., are animals of transport and travel in these countries, and of necessity must be more liable to infection than the cattle which the natives know how to keep in districts, or parts of districts, where they are most likely to survive. Moreover, it is not impossible to imagine that the cattle in these regions may have developed, in common with the wild animals, a relative immunity or refractoriness to *T. brucei*. It is hardly necessary to enter into any further considerations of this nature.

Type 2. *T. evansi*, affecting camels from Kordofan and Erkowit. In classing some of our known strains under this heading we are on less debatable ground than is certainly the case with two of the other types. Its general host here is most undoubtedly the camel, it is rarely dimorphic, and its morphology, as will be shown in detail, approximates to the *evansi* type ; moreover, its animal reactions resemble those of the Indian Surra.

Type 3. *T. nanum* or *pecorum*. This form has been found by Balfour to occur in epidemic form in the Shilluk cattle, and Drew came upon a similar type in goats near Ragaa in the Bahr-El-Ghazal. A specimen from the first of these sources was named *T. nanum* by Laveran, from films sent him by Balfour, in 1905. Wenyon, in the Third Report, speaks of *T. nanum* as occurring among mules and donkeys in the Bahr-El-Ghazal, and describes under this heading two main types with an intermediate form. A study of his article has led us to the conclusion that he was dealing with some other kind of trypanosome, because, for one thing, uniformity of morphology is considered by us to be an outstanding characteristic of this species. The reason that *T. pecorum* has been included in the nomenclature of this type is the strong morphological similarity between some specimens obtained from the cattle and goat infections, and the *T. pecorum* described by Bruce and others in the communication to the Royal Society, of June, 1910.

A possible error in classification

Type 4. *T. vivax* or *cazalboui*, from cattle in Kassala Province. This trypanosome, considerably shorter than *T. evansi*, is so typical on account of its extraordinary motility and its animal reactions, that, for this type, differentiation is simple. The question of the distinction, if any, between *T. vivax* and *cazalboui* will not be entered into here.

Methods of differentiation

We shall now briefly discuss in general the means which are at present at our disposal for differentiating trypanosomes, and it is here urged that some settled plan of procedure on the part of all observers would go far to prevent the inundation of scientific literature with the profitless discussions which arise round the nomenclature of similar type trypanosomes occurring in various parts of the globe. We have, at present, five possible headings, of varying importance and usefulness, under which classification may be attempted.

These are :—

1 Morphology

2 Animal inoculations and reactions

3 Culture

4 The question of the carrier

5 Reaction to drugs

These headings will now be gone into, a few words also being said about what is known as *cross-inoculation*, and it will be seen that by an application of some or all of these means a trypanosome can be classified with a reasonable amount of certainty, but it must at the outset be made plain that a certain degree of simplicity and definiteness is essential for satisfying the clauses under these headings, for as surely as the methods of identification are made numerous and complicated, so surely will the risk of unnecessary division of types increase. Not only are the relative values of the above headings of themselves far from being equal, but they also vary in value as a means of distinction for the different trypanosomes.

Morphology

In the first place we have *morphology*, and this, under some circumstances, is absolutely straightforward; for instance, no one seeing a slide of *T. nanum*, from general morphological considerations, would confuse it with *T. evansi*; but in most cases, and for trypanosomes of a somewhat similar structure, something more definite is required, and

this we have in Bruce's system. Under this system the trypanosomes are drawn at a given magnification (2000 diameters) and they are measured in a uniform way (*i.e.* with calipers down the body keeping the points of the instrument rigidly in the middle) from films, if possible, fixed and prepared in a uniform fashion—osmic fixation, Giemsa staining.

Bruce's system of measurement

From numbers thus drawn and measured the average size is obtained, and a maximum and minimum length is stated also, charts giving curves of the distribution, by percentages in respect to length, are prepared and the type thus graphically illustrated.

We have here, then, a definite and simple method of morphological classification which, following Bruce's system, is being carried out in these laboratories, and from which clear statements of results can be obtained.

It must be remembered that in this method there are some pitfalls and some fallacies, but they can readily be avoided or taken into account, as, for instance, in the comparison of trypanosomes from different animal sources. A comparison of tables from the measurements of trypanosomes from a common laboratory stock animal, such as the rat, is preferable to the indiscriminate study of the relation of trypanosomes in different classes of animals.

It is well known that the inoculation of the same strain of trypanosome into various animals is productive of certain differences in measurement, hence it is unwise to compare new species without taking this into account. In these laboratories the most convenient animal is the gerbil, but in general, any of the small rodents is, as a rule, a suitable animal. So much for morphological methods, on the whole simple and workable.

Animal inoculations and reactions

In *animal inoculations and reactions* we have another exceedingly useful method of distinguishing trypanosomes, but one that must be used with a somewhat open mind, for we know from laboratory experiments that, by certain methods of transmission, we can infect animals which have proved refractory to the original strain, and we can do this in a simple manner. Also, one sees how readily a strain, owing to laboratory transmissions, sometimes varies in character and virulence. We are thus led to keeping our distinctions, as far as animal reactions go, on broad general grounds, and to avoiding the error of allowing overmuch importance to the refractory power or susceptibility of occasional animals. Pathological changes in animals should also be viewed in the same manner; for instance, opacity of the cornea is not seen in every case of Surra in dogs.

Cultural methods

Cultural methods. These have not proved of very great value, for beyond the point that various observers have been able to say that a certain trypanosome can be "grown readily" or "cannot be grown" we can arrive at few more subtle distinctions; however, so far, these methods do help us. But here again, the possibility of a trypanosome being easily growable, or the reverse, according to its more immediate host environment, must not be forgotten. Also, the preparation of media is dependent on individuals and different supplies of blood serum—points well illustrated by the fact that some observers have been able to grow known strains while others have failed.

These conclusions will make us chary of jumping to definite conclusions under this heading.

The *question of carrier* is one probably of more importance in practical bionomics than in differentiating trypanosomes, still it is also useful in this connection; but an acquirable suitability of carrier is as understandable as an acquirable susceptibility of host.

Reaction to drugs

Reaction to drugs, is a method at present of the most questionable importance and of which little can be said except that it is unsatisfactory. Two drugs are being used here in this connection, metallic antimony and Ehrlich's "606," but from experience with various antimony and arsenic compounds, little can be hoped as yet from this method.

This will readily be understood if ability to produce antimony-fast and arsenic-fast strains be acknowledged, and the great want of uniformity of result between the action of a similar dose of a drug on similar animals, which have been infected at the same time, be known. Finally, as to Laveran's *cross-inoculation* method, which depends on the fact that animals naturally recovered from a trypanosomiasis cannot again be infected with the same disease though still susceptible to other strains, its application is necessarily limited; for unless a large number of "immunes" be kept up, a considerable time is required to prepare an immune animal. This difficulty is increased by the fact that animals in which trypanosomiasis is an acute disease die when inoculated, while those in which it is chronic, live on, presenting us with the difficult problem of accurately fixing the time of recovery. Also, unless the experimental animal is to some extent resistant to the trypanosome under investigation, it is lost to the laboratory.

Cross-inoculation

The method must be looked upon as cumbersome and not easy to carry out.

In the writer's experience with *T. evansi*, animals cured by drugs are as susceptible to a secondary inoculation of the original trypanosome as they were to the primary, a point giving rise to more theoretical fallacies. On the whole, therefore, the method does not appear suited to general tropical laboratory work.

The introduction to this short study of the trypanosomes of the Anglo-Egyptian Sudan was written with a view to make clear to the reader the general extent of our present knowledge on the subject, and also to convey, in general, an impression of the main principles which have been observed in making this study.

If the second object has been attained, the importance attached to the various phenomena recorded in connection with our four types will be readily understood, certainly at least as far as the work of classification goes.

A confession of faith on the part of a writer of a scientific article would go far sometimes to rendering his writings intelligible. An ignorance of the axioms and postulates governing his observations renders them often needlessly obscure.

The general acceptation of Koch's postulates must have prevented endless discussions on the pathogenic bacteria, for they make clear an observer's view-point.

As no such general postulates are forthcoming on the subject of protozoal infections, it is pleaded that there is justification for indicating a point of view before entering into the treatment of a subject like trypanosomiasis, of which the manifestations are as varied as the manifestations of grace.

The four types have been described roughly in the order of what is at present considered to be their relative economical importance; and the amount of work done on them follows closely in the same sequence.

Type 1

T. brucei or *pecaudi* (Plate I., figs. 1–2)

The distribution of this trypanosome is certainly of a very extensive nature and probably affects numerous species of animals. Its occurrence as affecting more particularly horses, mules and donkeys in the Bahr-El-Ghazal and on the White Nile, which has already been mentioned, is far from leading one to suppose that it is limited to these regions or to these animals. The outstanding characteristics of the type, as observed here, are its well-marked and persistent dimorphic character and its virulence towards dogs and the smaller animals. We have, as a point of interest, recently received additional evidence that it occurs in the former as a natural infection.

(*a*) *Living and unstained.* An actively motile trypanosome occurring in two easily recognisable forms, the long slender forms with a free flagellum and short stouter forms

Morphology

with no free flagellum. There are, of course, gradations between the two, and this distinction, though very convenient, is not intended to be arbitrary. An interesting point, however, has been noticed here during observations made on the disease in gerbils. It has been found that, in a commencing infection, the long slender forms are the first to appear and the stout forms are not found till the infection is well advanced; further, these forms are very much in evidence in the closing phases of the disease.

(*b*) *Fixed and stained.* In general, the films were stained here simply by the ordinary Leishman method. Osmic acid fixation with Giemsa stain was also used. Studied in this way, the two forms mentioned above are very apparent but, as stated, there is no distinct line of demarcation between the two, intermediate forms being easily recognised.

Length.—The measurements are made, as explained in the introduction, after Bruce's principle, many of the tables below being compiled directly from measurements so kindly undertaken and results obtained by Colonel Sir David Bruce in his London Laboratories, and to whose courtesy and help we are so much indebted in preparing this paper.

Table 1. Measurements of Trypanosomes of Type 1 (*T. brucei*).

Date	Animal	Method of fixing and staining	In microns			Remarks
			Average Length	Maximum Length	Minimum Length	
January 27, 1906 ...	Rat ...	—	22·9	31·0	14·0	Measurements by Colonel Sir David Bruce, 1910
February 2, 1908 ...	Gerbil ...	—	20·7	28·0	16·0	
February 11, 1908 ...	Gerbil ...	—	21·2	33·0	16·0	
November 20, 1908 ...	Monkey 2	—	16·8	25·0	14·0	
December 12, 1910 ...	Gerbil ...	Leishman ...	22·9	37·0	16·0	Measurements Khartoum, December1910–January 1911
December 21, 1910 ...	Gerbil ...	Leishman ...	21·4	27·0	15·0	
January 1, 1911 ...	Gerbil ...	Leishman ...	26·4	34·0	18·0	
	Gross average		22·2	30·7	15·6	

In comparing this table with Bruce's own measurements of *T. brucei* of the original Zululand strain discovered by him in 1894, we are at once struck by the resemblance.

	Average Length	Maximum Length	Minimum Length
Trypanosome Type 1, Khartoum	22·2	30·7	15·6
T. brucei, Zululand, 1894	22·8	35·0	13·0
T. brucei, Uganda, 1909	23·6	34·0	15·0

In the above table, twenty trypanosomes were measured in each case, and from these the average length was obtained, the length of the longest and shortest form being also given.

It must again be emphasised that these tables have their value rather as a synthetic whole than for detailed analysis, and that discrepancies in detail must not be allowed an undue importance. For instance, the table shows a variation in maximum lengths between 27 and 34 microns in the last two cases. We have mentioned above the varying

prevalence of the slender and the stout forms, and this alone, taken with the fact that the trypanosomes are measured just as they come on the slide, will save one from the pitfalls of detailed critical analysis. Following still in Bruce's footsteps we give charts showing the curves of the distribution by percentages in respect to length. Chart 1 is a reproduction of a chart prepared by Colonel Sir David Bruce in London in 1910. Chart 2 was compiled in Khartoum, and on this chart is shown the striking resemblance between the curve obtained here and the curve distribution of the strain of *T. brucei* from Uganda, 1909, as depicted in the *Proceedings of the Royal Society*, B., Vol. 83, 1910 (Colonel Sir David Bruce and others).

CHART 1

Type 1.—Chart compiled by Colonel Sir David Bruce, 1910

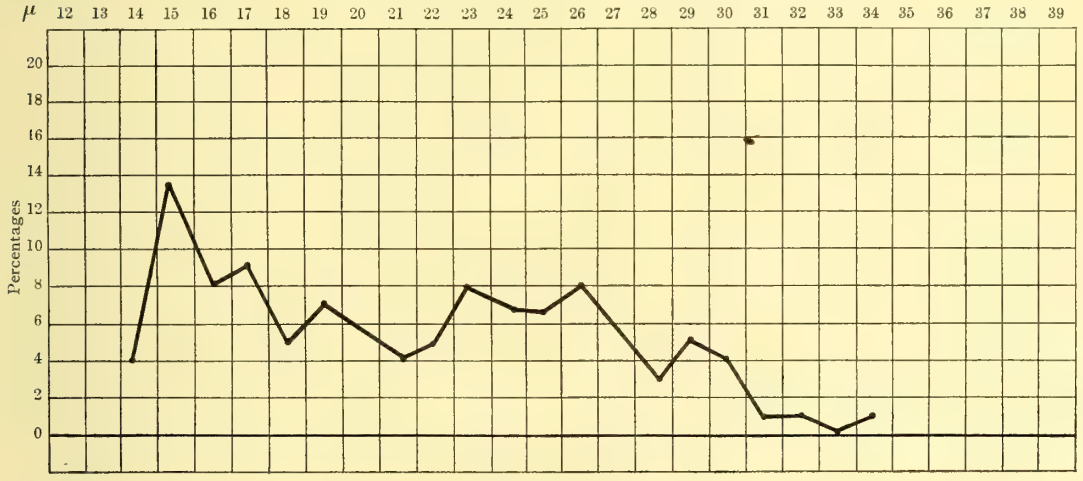


Fig. 12.—Curve distribution by length of Type 1

CHART 2

Type 1.—Chart compiled in Khartoum, 1911

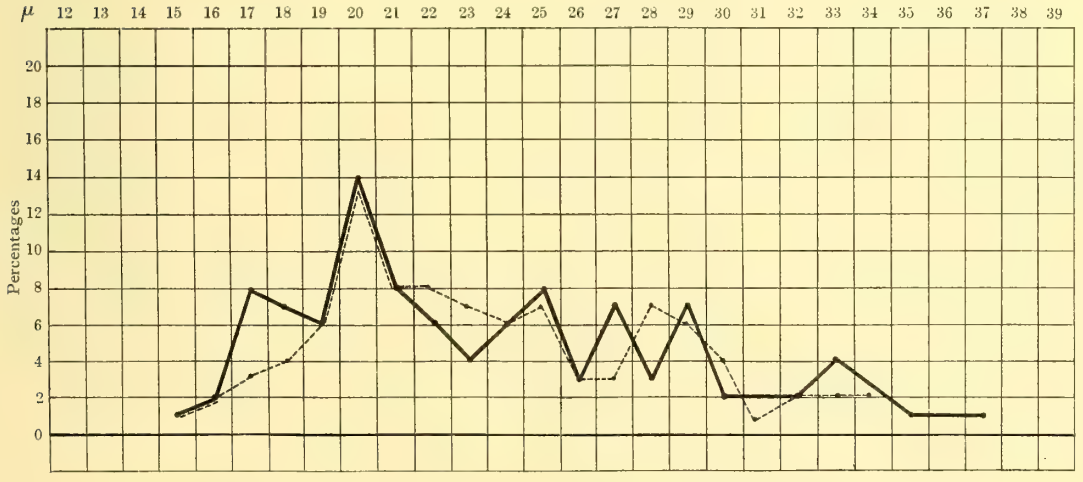


Fig. 13.—Curve distribution by length of Type 1. Dotted Line—*T. brucei*, Khartoum. Solid Line—*T. brucei*, Uganda
N.B.—The Khartoum Curve was compiled from 150 measurements

Added interest is given to these curves if they are compared with the two charts given of Khartoum Type 2—*T. evansi* (*page* 51).

Breadth. The long slender forms average from 1 to 2 microns; the short stout forms from 2 to 3 microns.

Cytoplasm. The cell contents at times show well-marked granules but, as a rule, stain a clear pale blue.

Nucleus. Is situated about the middle of the trypanosome and is oval or kidney shaped in the long forms and round or oval in the stout forms.

Micronucleus. Small and round; situated from 1 to 2 microns from the posterior extremity.

Undulatory membrane. Shows well-marked folds and undulations.

Flagellum. Free in the long forms and measures on an average 6 to 7 microns.

Animal Inoculations and Reactions

The following tables show the laboratory animals susceptible to this strain. They have been compiled from the records in the Laboratories of the Gordon College. It will be observed that the trypanosome is fairly virulent to most of our stock animals.

DOGS

Experiment Number	Duration of the disease	Remarks
X 6	23 days	Corneal opacity, pericardial effusion, enlarged spleen
X 7	28 days	,, ,, ,, ,, liver and spleen enlarged
X 11	13 days	,, ,, ,, ,, ,, ,, ,,
X 14	25 days	,, ,, ,, ,, ,, ,, ,,
X 18	14 days	Pericardial effusion, liver and spleen enlarged
A 1	34 days	,, ,, ,, ,, ,,

Average duration of the disease in dogs = 23 days

MONKEYS

Experiment Number	Duration of the disease	Remarks
X 8	16 days	Spleen and liver enlarged
X 13	21 days	,, ,, ,,
X 15	11 days	,, ,, ,,
X 22	36 days	Spleen moderately enlarged
X 26	37 days	Spleen enlarged, ulcer in cæcum
X 30	20 days	,, ,, ,, ,,
X 35	26 days	Spleen little enlarged, corneal opacity
X 37	32 days	
X 40	31 days	
X 41	15 days	
X 48	17 days	Ulcerated stomach
X 49	44 days	
X 50	18 days	Spleen and liver enlarged
X 51	24 days	,, ,, ,,
X 52	16 days	,, ,, ,,

Average duration of the disease in monkeys = 24 days

GERBILS

Experiment Number	Duration of the disease	Remarks
X 19	21 days	Enlarged spleen
X 21	16 days	,, ,,
X 23	8 days	,, ,,
X 25	15 days	,, ,,
X 27	10 days	
X 31	6 days	,, ,,
X 32	16 days	
X 33	9 days	
X 34	12 days	
X 39	34 days	
X 42	6 days	
X 43	21 days	
X 44	20 days	
X 45	24 days	
X 46	16 days	
X 47	22 days	
A 3	7 days	
A 4	17 days	
A 5	8 days	
A 7	20 days	
A 8	32 days	
A 10	16 days	
G 11	23 days	
G 12	8 days	

Average duration of the disease in gerbils = 15 days

In *rabbits* experiment X 17 gives 39 days as the duration of the disease, baldness and blepharitis being a feature of the malady. The conclusion arrived at here is that in rabbits the disease is of a somewhat chronic type.

In *jerboas* the disease is acute, killing the animals in about 7 days.

Guinea-pigs and *rats* are also susceptible to this type of trypanosome.

3. *Culture.* This trypanosome has been cultivated repeatedly in these laboratories by Mr. George Buchanan, whose notes on this subject will be found at the end of this paper. In general it may be stated that it grows readily and is comparatively easy to cultivate.

4. *Carrier.* No laboratory experiments have been carried out here. *Glossina morsitans* and a great variety of biting flies abound in the districts where it appears to be endemic. At present we may perhaps regard *G. morsitans* as its carrier.

Drug-reactions in gerbils

5. *Reaction to drugs.* Some experiments have been carried out with disease produced in gerbils by this trypanosome. This animal has been selected as the course of the disease is an acute one, the trypanosome being constantly in the blood till death, and the fallacies due to dealing with a chronic disease in which the trypanosomes appear and disappear of their own accord, are avoided. One dose of metallic antimony in sterile olive oil (3 to 5 minims) causes complete clearance from the blood of all trypanosomes in from 2–3 hours. Recurrence takes place after several days but the antimony acts with great trypanocidal vigour.

One dose of Ehrlich's "606" given in the same way causes a diminution in the number of trypanosomes in 24 and 48 hours and probably does not postpone the end for more than 2 or 3 days—it is relatively weakly trypanocidal. The exact amount of antimony and "606" in the dose is not here specified as it has been found by the writer from experiments performed both here and in London that probably only a small proportion of the drugs thus injected is actually absorbed by the body and thus rendered available for trypanocidal effects.

I do not propose to enter into a discussion as to whether Mr. Buchanan's work on the developmental and intra-corpuscular forms as found by him will necessitate a change of nomenclature in this type or not. His work on this point is very interesting and will be found in an article by itself following this paper. At present, however, I may say that I do not think we have sufficient grounds for embarking on fresh nomenclatures.

Type 2

T. evansi (Plate I., fig. 3)

The laboratory strains here of this trypanosome have been obtained from camels but there are some grounds for believing that mules and horses are at times affected. The disease is, as far as can be ascertained, probably widely distributed in the Sudan desert country where the camel is used. Its recognition by us as affecting camels in such widely separated districts as Kordofan and the Red Sea Province gives colour to this supposition.

Morphology

(*a*) *Living and unstained.* An actively motile trypanosome of a strikingly monomorphic type, no markedly noticeable variations in length occurring in the unstained parasite, an observation which is confirmed by measurements and study of the stained specimens.

(*b*) *Fixed and stained.* It is characteristically a long slender trypanosome with a well-developed flagellum, and the posterior end is definitely seen to be what is called "pike-headed." It certainly closely resembles the classical picture of the Trypanosome of Indian Surra.

MEASUREMENTS OF TYPE 2 (*T. evansi*)

Date	Animal	Method of fixing and staining	In microns			Remarks
			Average Length	Maximum Length	Minimum Length	
January 6, 1908 ...	Dog ...	—	24·4	29·0	20·0	Measurements by Colonel Sir David Bruce, 1910
January 16, 1908 ...	Rabbit ...	—	22·0	26·0	19·0	
December 13, 1908 ...	Camel ...	—	23·1	26·0	20·0	
January 25, 1909 ...	Dog ...	—	23·2	26·0	22·0	
December 20, 1910 ...	Gerbil ...	Leishman ...	23·0	27·0	17·5	Measurements Khartoum, December 1910–January 1911
January 2, 1911 ...	Gerbil ...	,, ...	24·5	29·0	21·5	
	Gross average		23·7	27·2	20·0	

This table is prepared in the same way as the preceding tables, and in it the close resemblance of the six series of measurements quoted here is very striking, when it is remembered that the strains which they represent have been obtained from different sources.

Two charts are here given showing the distribution by percentages in respect to length of this trypanosome; one was prepared by Colonel Sir David Bruce in London, and the second prepared in Khartoum.

CHART 3

Type 2—Chart compiled by Colonel Sir David Bruce, 1910

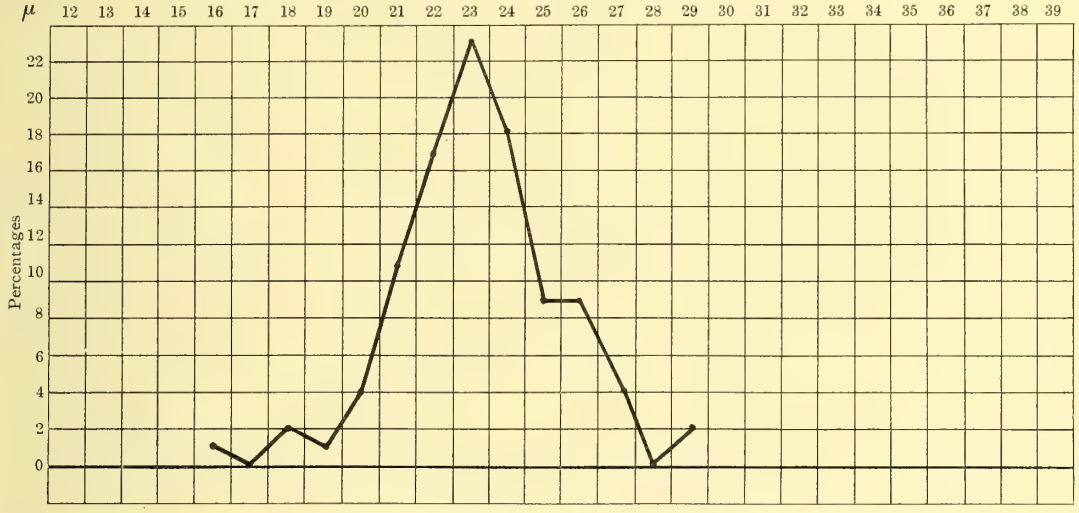


Fig. 14.—Curve distribution by length of Type 2 *(T. evansi)*

CHART 4

Type 2—Chart compiled in Khartoum, 1911

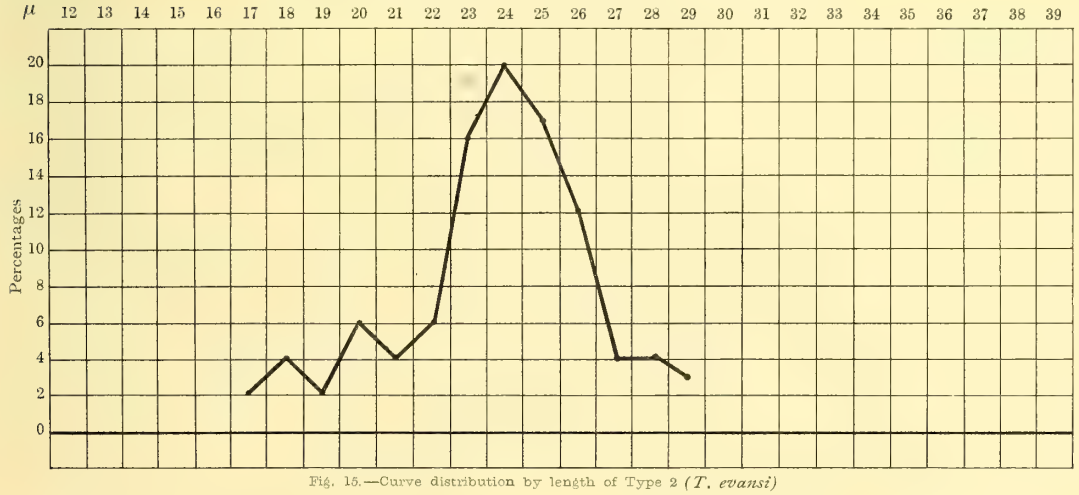


Fig. 15.—Curve distribution by length of Type 2 *(T. evansi)*

These two charts are very similar, and, as mentioned above, make a striking contrast with the charts showing the curves of Type 1 and the *T. brucei* of Uganda.

Breadth. This trypanosome varies from about 1·5 to 2·5 microns in breadth.

Cytoplasm. The cell contents do not show the granules as well marked as seen at times in Type 1.

Nucleus. Is situated in the middle of the trypanosome and is oval or round in structure.

Undulatory membrane. Well developed and shows well-pronounced folds and undulations.

Flagellum. Free in all forms found, and as such, measures from 5 to 7 microns.

Animal Inoculations and Reactions

GERBILS

Experiment Number	Duration of the Disease	Remarks
G 1	16 days	
G 2	over 25 days	
G 3	over 12 days	
G 4	10 days	
G 5	11 days	
G 8	93 days	Liver and spleen enlarged
G 9	over 60 days	
G 10	over 35 days	

It will be seen from these results that, though this trypanosomiasis sometimes runs an acute course in gerbils, it also at times occurs as a chronic type, the trypanosomes disappearing from the blood for several days at a time, to reappear and swarm again with another subsequent disappearance.

In rabbits the disease is chronic, blepharitis and baldness being symptoms, and the disease resembles in every way in this animal the effects produced by Surra.

In guinea-pigs the disease is chronic and shows no marked characteristics. Dogs are susceptible and die with marked splenic enlargement, the symptoms appearing to be indistinguishable from Surra.

3. *Culture.* All experimental efforts, so far as culture goes, in these laboratories have been failures. The cultivation evidently presents more difficulties than that of Type 1, certainly as far as our present methods go.

4. *Carrier.* In both the Red Sea Province and Kordofan the natives attribute the disease to the agency of the seroot fly. As far as one can definitely say anything on this heading in the first-named of these regions, *Tabanus sufis* is the causal agent of transmission; in the latter *Tabanus tæniola.* In both places several species of *Tabanus* exist, and species of *Hippobosca* are also found.

5. *Reaction to drugs.* The disease in the laboratory animals available at present being of a chronic nature, it has been deemed unsatisfactory to quote any experiments with this trypanosome as the chronicity of the disease tends to make the results misleading.

It is interesting here as a footnote to this section to state that Sir John McFadyean is not quite in agreement with the writer of this paper on the close resemblance of our Type 2 trypanosome to that of Indian Surra. He states in a letter to the Director of these laboratories "I have still the camel trypanosome running. I do not think that it corresponds with the Indian *T. evansi*, but it is a very hard matter to distinguish between some of the trypanosomes from morphological characters."

Type 3

T. pecorum or *nanum* (Plate I., figs. 5, 6, and possibly 7)

Unfortunately, no strain of this trypanosome has been available in these laboratories in the last year, so the consideration of this type must be a short summary of investigations performed some time ago and a record from work on stained specimens. This type has been carefully described by Balfour in the Second Report, in discussing trypanosomiasis among the Shilluk cattle.

Up till recently *T. nanum* has been considered a distinct entity, *i.e.* a small trypanosome affecting cattle and possibly mules; cattle may either die or recover and so far as is known the parasite is conveyed by *G. morsitans*. More extended investigations go to show that *T. nanum* affects other animals in the Sudan, or at least that there are types very closely allied to it which affect horses, goats and sheep.

A great deal more work is required to elucidate these types, especially with regard to the susceptibility of laboratory animals. This will be referred to under "animal inoculations and reactions" where the main difficulties of arriving at a conclusion are further entered into. We shall now follow the same system of description as with our first two types, but it must be remembered that we are fully alive to the fact that we may be dealing with the characteristics of possibly more than one variety of trypanosome.

Morphology

(*a*) *Living and unstained.* This trypanosome has never been observed in fresh blood by the writer but an extract from Balfour's description is here given verbatim. "The trypanosome is a small one. It is not very active in fresh films and I have never seen one traverse the whole field of the microscope. The motion is undulating combined with a vigorous lashing to and fro of the anterior part of the body which tapers to a very tiny flagellum. Rippling and what may be termed spreading movements have been observed. The parasite advances usually with the narrow end in front but this motion is often reversed. A fact that is very noticeable is that the trypanosome tends to adhere to the red blood corpuscles. Even in a thin field this is seen, the parasite seeming to take a delight in butting and boring at the erythrocytes. Frequently it gets beneath them and is lost to view, the agitation it produces being the only clue to its presence." This graphic description is exceedingly interesting when compared with Bruce's description of the *T. pecorum* of Uganda which is also given verbatim. "This trypanosome, when observed in a preparation of fresh blood, is seen to remain at or near the same spot in the field, that is to say it is non-translatory. It is, however, active and restless, the body quivering rapidly, and the undulating membrane and flagellum keeping up a constant vibratory motion. As a rule it moves with the flagellum end in front. The contents of the cell are homogeneous, except for a vacuole at the posterior extremity.

Descriptions by Balfour and Bruce compared

"A marked characteristic of this species is that it exhibits alternating periods of quiescence and activity. When quiescent it is usually invisible as it has a habit of burying itself under small collections of red blood corpuscles."

One cannot but be struck by the resemblance of these two descriptions, the one of the trypanosome of the Shilluk cattle and the second that of *T. pecorum* in the domestic animals of Uganda.

(*b*) *Fixed and stained*

Length. The tables of lengths here given are all prepared by Col. Sir David Bruce from slides given him by Dr. Balfour, but do not include the original type strain found in cattle. As there is doubt as to what strain of trypanosome we are dealing with, the provisional nomenclatures, as supplied by Col. Sir David Bruce, are here given above the different slide measurements.

Trypanosoma pecorum or *nanum* (and *vivax*?)—(BALFOUR'S TRYPANOSOME OF GOATS)

Date	Animal	Method of fixing and staining	In microns			Remarks
			Average length	Maximum length	Minimum length	
April 10	Goat ...	Leishman	13·5	16·0	12·0	Double infection
Trypanosoma nanum (BALFOUR'S TRYPANOSOME OF LANDON'S PONY)						
February 3, 1910 ...	Horse ...	Leishman	12·6	15·0	10·0	
Trypanosoma? BALFOUR'S TRYPANOSOME OF WENYON'S DOG						Measurements by Colonel Sir David Bruce, 1910
April 1, 1906	Dog ...	Leishman	16·2	18·0	13·0	
Trypanosoma? BALFOUR'S TRYPANOSOME OF SHEEP (BAHR-EL-GHAZAL)						
—	Sheep ...	Leishman	15·7	18·0	13·0	

With reference to these slides, in the first case where *vivax* is mentioned an occasional trypanosome was found that varied from the general type of the infection, and this led Colonel Sir David Bruce to conclude that there was the possibility of a mixed infection. This type was not included in the drawing from which the measurements were made.

With regard to the second slide, this pony came from Uganda where it was possibly infected.

With regard to the dog and sheep slides Colonel Sir David Bruce supplies the following footnote :—

"These trypanosomes look very alike. They are most closely related to *Trypanosoma pecorum*, but are larger. It may be a new species but would require to be studied further in different animals before an opinion could be given."

It may be stated that the Wenyon dog strain was originally derived from a heifer on the Sobat, and it appears probable that this monomorphic strain is quite distinct from the so-called dimorphic *T. nanum* which the same observer found in mules in the Bahr-El-Ghazal.

With regard to the other dimensions the descriptions given below apply in general to all the above specimens.

Breadth. The average breadth is from 1 to 2 microns.

Cytoplasm. Generally clear and staining a light blue in some specimens ; well-marked granules are apparent.

Nucleus. Oval in shape and is situated somewhere about the centre of the body.

Micronucleus. Small and round and situated near and often apparently at the posterior extremity.

Undulatory membrane. Fairly well developed.

Flagellum. As mentioned on the previous page there is no free flagellum, or if there is, it is extremely short.

As a matter of comparison one might refer the reader to the original *T. nanum* measurements by Laveran and Balfour, as stated in the Second Report of these

Laboratories (*pages* 119, 120). While these were not done according to Bruce's method they are sufficiently accurate for all practical purposes. The fading of the old films has rendered satisfactory re-measurement impossible. We have, however, made use of the chart showing curves of distribution in respect to length of *T. nanum*, Uganda, 1909, and Balfour's *T. nanum*, Sudan, 1904, as represented in the *Proceedings of the Royal Society*, B., Vol. 83, 1911. (Col. Sir David Bruce and others.) The second curve mentioned was prepared from slides supplied by Dr. Balfour in the summer of 1910.

Type 3. Chart copied from the *Proceedings of the Royal Society*, B., Vol. 83, 1911.

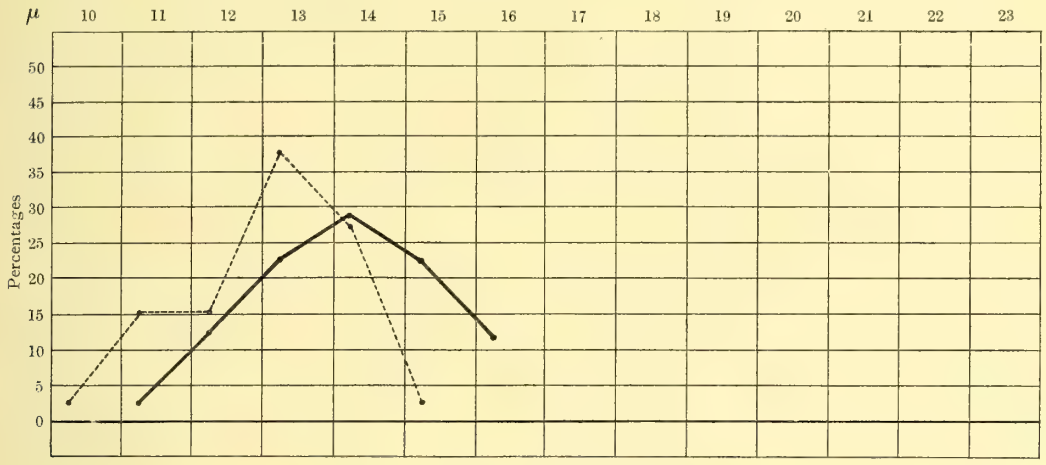


Fig. 16 {Dotted line—*T. nanum*, Sudan, 1904
{Solid line—*T. nanum*, Uganda, 1909

Animal Inoculations and Reactions

The following experiments were performed by Dr. Balfour, and are detailed by him in the Second Report of the Laboratories (*pages* 120, 121):—

Experiment Number	Animal	Result
X 1	Monkey	Negative
X 2	Rabbit	,,
X 3	Rabbit	,,
X 4	Monkey	,,
X 5	Dog	,,

These inoculations were carried out with the trypanosome from the same cattle strain of which the slides were sent to Laveran, who gave this trypanosome its title of "*nanum*."

These experiments are, as Dr. Balfour himself confesses, quite inadequate to furnish any exact idea of the pathogenicity of this trypanosome to laboratory animals.

Attention is drawn strongly to this point because monkeys, dogs, rats and mice have all been shown to be susceptible to *T. pecorum* of Uganda; and if, as it would appear above, these animals prove insusceptible to *T. nanum* on further experiment, we shall be forced to create new types or at least make one sub-division. However, until we can carry out more detailed and extended experiments on these points we shall class these possibly various strains of trypanosomes under the heading *T. nanum* or *pecorum*, regarding the morphological resemblances as sufficient justification for so doing.

3. *Culture.* No experiments have as yet been undertaken in this country to attempt to cultivate any strain of this type.

4. *Carrier.* The carrier of this trypanosome is not definitely known, biting flies and ticks have been suspected; they both abound in those regions where this disease is prevalent.

5. *Reaction to drugs.* No experiments have been performed.

Type 4

T. vivax or *cazalboui* (Plate I., fig. 4)

No living strain has as yet been available for investigation in these laboratories. Dr. Balfour has described the occurrence of this type under the heading of "Cattle Trypanosomiasis of Kassala" in the Third Report. From a consideration of its morphology in the stained specimens which have been available, we have decided to class this type as above. As so little is known we shall give its characteristics in all brevity.

Morphology

In general, resembles very closely the type specimens of *T. vivax* as depicted by various observers, showing the well-known characteristic shape.

Length. Variations in length are apparently not marked.

TYPE 4. *T. vivax* or *cazalboui*

Date	Animal	Method of fixing and staining	Average length in μ	Maximum length in μ	Minimum length in μ	Remarks
January, 1907 ...	Cattle ...	—	19·1	21	18	Measurements by Colonel Sir David Bruce, 1910

Breadth.—From 2 to 2·5 microns. *Cytoplasm.*—Clear staining cell contents, rarely granular. *Nucleus.*—Long and oval, situated towards the anterior extremity. *Micronucleus.*—Large, round, and terminal or sub-terminal. *Undulatory membrane.*—Unfolded and well developed. *Flagellum.*—Free, from about 3 to 5 microns.

Inoculations

Animal inoculations are known to affect cattle and supposed to be very virulent to sheep and goats in Abyssinia. The insusceptibility of smaller animals to this type has been the cause of our difficulty in obtaining a living strain.

3. *Culture.* This species is said to grow readily so that we are in hopes of soon being successful in this direction.

4. *Carrier.* Supposed to be a *Tabanus* or *Hippobosca.*

5. *Reaction to drugs.* Nothing is known.

We have now completed our present classification into four types, but, doubtless, we shall shortly find need for amplification and modification in the classes as worked out here.

Captain Archibald is, at the time of writing this paper, proceeding to the Lado with the object of obtaining and working on the strains of that district, and, as he is taking up the floating laboratory and is well provided with experimental animals, we have good reason to hope for the most fruitful results.

In conclusion, I have to thank most sincerely Colonel Sir David Bruce, C.B., F.R.S., A.M.S., for his extremely kind help and advice as to the treatment of the animal trypanosomiasis of the Anglo-Egyptian Sudan and for the use of his charts and measurements of which this paper is so largely made up, and Lady Bruce for the excellent Trypanosome Plate.

I am indebted to Major Standish O'Grady and Captain W. R. O'Farrell, R.A.M.C., for great assistance in making drawings from microscopic specimens and in other directions. Mr. George Buchanan's work in these laboratories speaks for itself in this Report.

PLATE II



G. Buchanan

1—3. Cultivation Forms of *T. brucei (pecaudi)*

1. Agglomeration of trypanosomes in 24 hours' culture
2. Young forms from a subculture of 15 days
3. Flagellate herpetomonad forms from 15 days' subculture

1—2. *Leishman Stain*
3. *Giemsa Stain*

× 2000 *diam.*

4—25. Developmental Cycle of *T. brucei (pecaudi)* in *Gerbillus pygargus*

4—12. Endoglobular forms in the spleen
13—17. Encysted forms in splenic pulp
18—23. Forms found in lung smear on sixth day after inoculation. Fig. 23 shows basal granule
24—25. Forms found in smear from axillary gland on seventh day after inoculation

× 2000 *diam.*

Some Observations on Trypanosoma Brucei? (Pecaudi?) and the Sudan Camel Trypanosome in Cultures

with a note on

Endoglobular and Developmental Forms of T. Brucei? (Pecaudi?)

by

George Buchanan

Senior Laboratory Assistant

Wellcome Tropical Research Laboratories, Khartoum

The cultivation of these trypanosomes was carried out mainly with a view to determine if their cultural features corresponded to those of *T. brucei* and *T. evansi* respectively, to which in many other respects they appear to be closely allied.

Technique

The culture media used were Novy and MacNeal's blood agar and Nicolle's modification of the same. The inoculations were made from the heart's blood of infected gerbils. Not more than two drops of blood from a capillary pipette were usually introduced into the small amount of condensation fluid, the tubes then being incubated at 22° to 25° C.

T. brucei (pecaudi)

The inoculations were made at a time when the trypanosomes were not too numerous and before the appearance of the swollen involution forms in the peripheral blood. It was found that cultures inoculated with blood showing a heavy infection, or after the animal had succumbed to the disease, were invariably failures, as in such cases there was a preponderance of degenerated trypanosomes which only further rapidly degenerated in the fluid. Technique

Nicolle's medium unfailingly showed motile trypanosomes up to sixteen days, while in Novy and MacNeal's blood agar no recognisable forms were seen after five days as a rule, with the exception of one culture which showed feebly motile trypanosomes up to the thirtieth day, and from which sub-inoculation was successful.

Appearance in Culture

The 24-hours' cultures showed numerous individual trypanosomes actively motile, while clumping in the form of rosettes was also much in evidence (Plate II., fig. 1). The tendency to agglomerate at this stage was very marked in all cultures, and such formation rapidly progressed on the union of two trypanosomes being established, others then appearing and uniting by their anterior ends. The numbers forming clumps averaged eight, although, in one instance, as many as eighteen were seen. Several were also observed dividing longitudinally at this stage. Cultures

The stained films showed trypanosomes in varying stages of degeneration. The flagella had disappeared in the presumably young forms, while others, corresponding to the swollen trypanosomes in the peripheral blood, were in an advanced stage of disintegration, assuming very eccentric forms with elongated and thickened flagella.

In the 48-hours' cultures, the number of trypanosomes was well maintained, but the clumps were not so numerous, and those seen presented a whorl-like formation. The trypanosomes in the stained films contained vacuoles and were rather granular in appearance.

From 72 hours onwards there was a progressive decrease in the number of trypanosomes, their movements being more rotatory in nature and very sluggish. The clumps disappeared entirely and those trypanosomes present on the eighth and sixteenth days, in both media, reacted very feebly to Leishman's mixture, staining a diffuse pale blue colour with no evidence of nucleus or flagellum.

Subculture successful

As mentioned on the previous page, subculture into Novy and MacNeal's blood agar was successful from a thirty-days' growth in the same medium. No forms were recognised in the subculture until about the fifteenth day, when young trypanosomes, long, thin, and of uniform thickness were seen, some possessing a terminal flagellum in active motion but not communicating the characteristic undulatory waves to the body, which never altered its regular outline. Typical rosette forms were also very numerous, the numbers composing which ranged from seven up to sixty. Some clumps consisted of dividing spherical and irregular forms (Plate II., fig. 2), while the larger masses comprised forms from small round types in the centre to almost fully-developed trypanosomes radially disposed at the periphery.

The blepharoplast and nucleus showed up pronouncedly in the stained films, and the protoplasm appeared vacuolated and granular in the young spherical forms, some of which were fused together with dividing blepharoplast and nucleus indiscriminately placed. In the more fully developed trypanosomes, short immature flagella were seen attached to their anterior ends, the blepharoplast in many cases being likewise situated anteriorly, thus presenting figures identical to the herpetomonad type of parasite (Plate II., fig. 3).

Appearance of a herpetomonad type in culture

This strain of *T. brucei (pecaudi)* is being still further successfully subcultured without difficulty, the forms most numerous in each succeeding subculture being those elongated trypanosomes with the terminal flagella which have been most in evidence since the second subculture.

The virulence of a twenty-two days' culture was tested on a gerbil, but the animal never became infected.

Remarks—Although the behaviour of this strain in culture is much like that of *T. brucei* in some respects, yet, from the numerous cultures made, one cannot say that it only grows *exceptionally* on culture media, upon which fact Novy and MacNeal lay stress. Primary cultivation proved comparatively easy in the sense that the trypanosomes maintained their motility and numbers moderately well in the original cultures, and this perhaps more so in Nicolle's medium provided no bacillary contamination ensued after repeated examination. It is true, however, that they did not multiply. Like *T. brucei* it certainly does grow very readily after the first subculture has been established and, in subsequent cultures, the trypanosomes multiply on an extensive scale.

T. EVANSI (CAMEL STRAIN)

Repeated attempts were made to cultivate this trypanosome from gerbil's blood in the above-mentioned media but with no success save in so far as the parasites were evident and remained motile up to about eight days, after which no forms could be recognised either in hanging drop or stained films. A few sub-inoculations were made, but none of them revealed trypanosomes at any period, the media remaining quite sterile for many weeks afterwards.

Difficulty in culture attempts with *T. evansi*

Even after 24 hours' incubation the cultures invariably showed a decided decrease in the number of trypanosomes, although those surviving were very actively motile. The formation of clumps was not marked at any period. In the stained preparations all the trypanosomes present appeared much thinner and decidedly degenerated, with

no flagella. Their motility became less active each succeeding day and, after the third day, no typical trypanosome forms could be seen, all having assumed small spherical or pear-shaped forms with long flagella attached. Some clumps of these forms were seen on the sixth day slowly performing rotatory motions but no flagella were visible, they having gradually shortened and finally disappeared. Experiments with *T. evansi*

Subcultures were usually inoculated on the fifth day when the round forms were present, but examination up to three weeks showed no recognisable trypanosomes.

The culture work with this trypanosome is very incomplete as variations in the proportions of blood and agar, advocated for other species, have not yet been tried, so that nothing definite can be said, meantime, regarding the success or failure in cultivating this strain. But the attempts made, their failure, and the forms met with up to a limited period, correspond very closely to the cultural behaviour of *T. evansi* described by Laveran and Mesnil.

Note on Developmental Forms of T. brucei (pecaudi) in the Internal Organs, Axillary Glands and Bone-marrow of the Gerbil (Gerbillus pygargus)

The transmission and maintenance of the trypanosome strains being under my care, ample opportunities were afforded of noting the progress of the disease induced by them in experimental animals and the structural changes in the trypanosomes themselves at varying periods. It was, however, mainly the cultural work associated with this trypanosome that led to further investigation of these developmental forms and thereby established the observations forming the subject of this note.

On beginning the culture work, inability to recognise if certain forms met with in cultures were either developmental or degenerative phases, suggested a study of the changes which the parasite undergoes, both in the body of the gerbil after death and in citrated blood, and the comparison of these changes with the forms found in Novy and MacNeal and Nicolle's media. With this in view, a gerbil was inoculated with infected blood and chloroformed on the fifth day after injection. Cultures from the heart's blood and smears from the various organs were made, all being examined at regular intervals for comparison. Light was thereby thrown on the point in question, but, in addition to this, attention was drawn to what undoubtedly looked like trypanosome forms in the red blood corpuscles in the spleen. Some of these were identical with those figured by Chagas in his paper on *Schizotrypanum cruzi*, which appeared at the time this work was being conducted. Trypanosome-like inclusions in the red cells of spleen

In order to confirm the observations made, a series of inoculations was carried out in gerbils.

In the first of the series, five gerbils were employed, these receiving subcutaneous inoculation in the flanks. In each of the two later series, however, it was found necessary to use eight gerbils at least, the injection being given intraperitoneally as recommended by Chagas. The amount of infected blood in citrate inoculated into each animal was 10 minims, all the eight forming one series receiving the injection at the same time. A gerbil of the series was then chloroformed on each succeeding day, beginning on the first day after injection. Films from the peripheral and heart's blood were taken, and smears from the lung, liver, spleen, bone-marrow and axillary glands were made, these being fixed and stained by various methods.

The intra-corpuscular forms appear from sixth to eighth day

As both the intra-corpuscular forms and those which possibly represent encysted stages appeared in the spleen on the fifth day in the first gerbil, a careful examination of the films in the first series up to that time was made, but none of the forms seen in the original slide were recognised. In the other series, however, examination of spleen smears from gerbils chloroformed on the sixth and seventh day respectively revealed both of the forms mentioned. These were also usually obtained in subsequent independent cases on the seventh and eighth day.

Morphology and Development in the Spleen

(*a*) *Intra-corpuscular.* No definite merozoite forms as described by Chagas were ever seen entering the red cells, and the small inclusion shown in Plate II., fig. 4, was the only appearance observed which suggested a merozoite. Speaking generally, the smallest intra-corpuscular forms met with took the shape of very small rings with two chromatin masses, and were about one-third the size of the red blood cell. Nearly all stages from this to a fairly mature trypanosome, contained within the limiting envelope of the red cell, could be traced, the intermediate stages in many cases being similar to those of *Schizotrypanum cruzi* (Plate II., figs. 4–12). This fairly mature trypanosome, however, possessed neither undulating membrane nor flagellum. Some of the more mature forms were coiled up or S-shaped, every part of them being wholly within the corpuscle. These varieties were not so numerous as the complete ring forms. The blepharoplast was not recognisable in all cases, but, when present, was usually situated at the thinnest part of the ring.

Ring forms

(*b*) *Extra-corpuscular.* Ring forms were also met with free in the plasma, and these impressed one as being possibly encysted and likewise showed a development similar to that seen in the red cell. Numerous small solid, encysted (?) forms appeared in the spleen on the fourth day in one gerbil (Plate II., figs. 13–17), and these, as a rule, were also present in other animals in the bone-marrow and axillary glands on and after the sixth day. The very small types appeared as spherical masses of densely-stained blue protoplasm, the nucleus in some being indicated by a small undefined violet mass, while the blepharoplast was detached.

Spherical bodies

Round the spherical body here described there existed, as a rule, at least when development was somewhat advanced, a clear area which gave the impression that the protoplasmic mass was lying in some form of vacuoloid space which was possibly surrounded by a limiting membrane. It was in connection with this membrane (?) that the blepharoplast was found, hence the use of the term "detached" (Plate II., fig. 15). It was found that, as the protoplasmic mass became larger, a clear area developed within it. This increased in size, the nucleus of the mass became more distinct, and finally the blepharoplast appeared gradually to approach the mass until it became part of it. Such a movement on the part of the blepharoplast is well-nigh inconceivable unless there was originally some connection between it and the main mass of protoplasm. Such, however, I have never been able to demonstrate.

Eventually the spherical masses became ring-shaped, resembling in every respect those met with in the red cells.

These stages were always observed within the vacuoloid space which enlarged to accommodate the requirements of the developing trypanosome. As in the red cells, forms almost mature were eventually found. These, which possessed free extremities, often assumed S shapes within a faintly defined capsule, the nucleus and blepharoplast being situated as in an ordinary trypanosome. Later observations showed that these

forms were invariably found free in the plasma of the spleen, bone-marrow and axillary gland several days previous to the appearance of the intra-corpuscular forms.

Forms found in Lung Smears

The probability of schizogony occurring in the lung as in the case of *Schizotrypanum cruzi* was borne in mind, but no definite schizonts were seen in any of the smears. Yet the forms figured by Chagas,[1] and described by him as parasites in the lung of vertebrates preparing for schizogony were exceptionally numerous in the lung of a gerbil on the sixth day. In fact they were the only forms present in smears from the lung at that time, but no suggestion of merozoite formation was ever observed. Some forms seen resembled the so-called latent bodies of *T. gambiense* described by Moore and Breinl,[2] and more recently by Fantham,[3] as occurring both in *T. gambiense* and *T. rhodesiense* infections. Their method of formation, however, seems to be somewhat different to that described by these authors. It is, perhaps, sufficiently indicated by the cycle of events shown in Fig. 17. In addition there was markedly evident the presence of individual spherical forms dividing into two, and occasionally three, separate bodies, each of which eventually assumes a trypanosome shape. As a result, large numbers of what were undoubtedly very young trypanosomes, each with a well-defined nucleus but without undulating membranes and flagella, were present in the lung smears.

Presence of many very young trypanosomes in lung-films



Fig. 17. {A. Apparent formation of non-flagellate body from trypanosome in lung
{B. Apparent metamorphosis of non-flagellate body into trypanosome in lung

No definite intra-corpuscular or free encysted forms were ever seen in the lung or peripheral blood. The former appeared exclusively in the spleen while the latter were found without fail in the spleen, bone-marrow and axillary glands.

The axillary glands showed an unending variety of young trypanosomes corresponding in the main to those described above in the lung, while the encysted stages were also very numerous.

A marked feature, perhaps worthy of note, was the constant presence of free chromatin granules in nearly all the smears from the second day onwards. These were apparently derived from the nuclei of disintegrating trypanosomes and possibly represented a first stage in development.

Free chromatin "granules"

[1] Chagas, C. (August, 1908), "Über eine neue Trypanosomiasis des Menschen." *Memorias de Instituto Oswaldo Cruz*, Vol. 1, No. 2.

[2] Moore, J. E. S. and Breinl, Anton (July, 1907), "The Cytology of the Trypanosomes." *Annals of Tropical Medicine and Parasitology*, Vol. 1.

[3] Fantham, H. B. (1910), "The Life-history of *Trypanosoma gambiense* and *Trypanosoma rhodesiense* as seen in rats and guinea-pigs." *Proceedings of Royal Society*, B. Vol. 83.

Notes on Human Spirochætosis

by

Captain L. Bousfield, M.A., M.D. (Cantab.), M.R.C.S., L.R.C.P., R.A.M.C.

late attached Egyptian Army

The following article is founded on observations derived from six cases of relapsing fever, which occurred amongst the troops at Khartoum in January, 1910, and from two cases seen in Cairo in March of the same year.

I believe relapsing fever had never been diagnosed in the Sudan before Captain A. G. Cummins, R.A.M.C., reported it in two cases at El Obeid, both being Egyptian soldiers who had just returned off furlough from Egypt.

Late in 1909 a case was found at the Civil Hospital at Khartoum, and this case apparently came from Egypt, as did five of the six cases treated at the Military Hospital, Khartoum.

Human spirochætosis probably is not endemic in the Northern Sudan, for it seems scarcely possible that it could have missed recognition, and everything tends to uphold the view that all the above-mentioned cases were imported from Egypt, where the disease is well known and causes considerable epidemics.

The important question arises whether the Egyptian may not be able to introduce this disease into the Sudan and so permanently infect a district at present apparently free, a question which has also to be faced with regard to bilharziosis, at least in certain localities.

Of the six Khartoum military cases the first was taken ill in Cairo twelve days after joining from his village, Ashmun Gurice, Moudiriah Manoufia, where it seems probable that he contracted it, for Captain R. G. Anderson, R.A.M.C., kindly made enquiries and found that there was an epidemic of this fever in a neighbouring village, Kafr-El-Gazzar.

History of the first case

The other recruits with whom he mixed were apparently healthy.

He was admitted to hospital in Cairo for six days with fever; probably this was his first attack as he asserted he had no fever in his village.

Very shortly after discharge he started for Khartoum and gave the history of being ill with fever most of the journey up country.

Arriving at Khartoum on January 1, he was admitted on the 2nd with fever, temperature 102° F., and suffered from a marked rigor shortly after admission.

Only a few spirochætes were found after a prolonged search.

The other patients were taken ill on January 3, 7, 9, (two) and 14 respectively, but were not admitted to hospital till January 5, 10 (two), 12 and 15.

The first five cases all occupied the same barrack-room at Cairo, all stated they were bitten by bugs while there, but only No. 1 was taken ill.

All occupied the same railway carriage on the way up to Khartoum and during this time must have been in intimate contact with each other.

On arrival at Khartoum the four yet unaffected went to separate barrack rooms, all of which probably harboured bed-bugs.

The main point of interest centres round the sixth and last case, the only one to contract the disease in Khartoum, for he had been resident in the Sudan for over a year.

This man did not occupy the barrack-rooms, but was the pack store-keeper, who issued clean clothes to the troops, taking the dirty ones and placing them in cloth bags ready for the wash.

It was found that he not only lived, but also slept, in the same room with these bags of dirty linen, prior to their being sent to the wash.

Every additional item of evidence on the transmission of this disease is of importance, and this last case seems to help to corroborate the view held by Dreyer, Kirton and Graham W. Smith in Egypt, and by Mackie in India, that lice, *Pediculi vestimentorum*, are the usual carriers of the disease from man to man.

Evidence as to probable "vector"

No definite evidence can be adduced from these cases, for, unfortunately, no *Pediculi capitis*, *vestimentorum* or *Phthirius pubis* were found in the infected cases, since, by the time the diagnosis had been made, their clothes had been sterilised and washed, following the general rule on admission to hospital.

Further, Dr. Andrew Balfour's experiments with *Pediculi vestimentorum* taken from other recruits coming up with these cases, failed, owing to the difficulty of keeping the lice alive.

Nuttall has proved that *Cimex lectularius* can convey the disease from mouse to mouse, and Schaudinn found spirochætes persisting in bugs up to 30 days, but it seems probable that the latter are not the usual transmitters of the disease.

The six Khartoum military cases were undoubtedly bitten by bugs in Cairo and in Khartoum, but no other cases occurred amongst the soldiers in Khartoum, though each case went to a separate barrack-room where bugs existed.

Dr. Balfour failed to find spirochætes in bed-bugs taken from the crevices in the wall near the bed of one of the cases.

The flea is considered not to convey the disease, though it is certainly food for thought that the disease is common in Egypt where fleas abound, while unknown in the Sudan, where this pest is, as far as my knowledge goes, practically never seen, though *Cimex lectularius* and *Pediculus vestimentorum* are common to both countries.

The case of the pack store-keeper seems to implicate *Pediculus vestimentorum* rather than the flea or the bed-bug.

Clinical picture

A brief summary of the clinical aspect of the disease is given.

The duration of the first attack varied from 2–8 days, while the relapses varied from 4–6 days.

One case probably had three relapses, one had two relapses, five had one relapse and one had only one attack of fever.

The fever usually reached its maximum or thereabouts during the first 24 hours with subsequent morning remissions.

The termination of all, except one, was by crisis with marked diaphoresis.

Between attacks, and subsequent to the last attack, the temperature was subnormal for some days.

The case having no relapse showed the highest temperature, 107·4° F., and the pyrexia only lasted two days.

The spleen was palpable in six cases, enlarged to percussion in one and apparently not enlarged in another.

Increase in size of the spleen was noted on several occasions during a relapse.

Seven complained of pain in the splenic region and two had spleens tender to palpation.

The liver was not enlarged in any case, but was tender and painful in two.

Anæmia was present in five cases, in two to a marked degree.

Epistaxis was noted in two cases, a definite rigor in one, herpes labialis in one and vomiting in two.

Prostration and weakness

Prostration and weakness were marked in all, but, with the subsidence of the fever, rapid recovery ensued in all but one debilitated case.

Headache, backache and a moist white furred tongue were seen in all, and three complained of pain and tenderness over the tibiæ.

Jaundice, eye affections, albuminuria and diarrhœa were never encountered.

The heart and the lungs were normal, though one case showed some congestion of the right base.

The two cases which were admitted in March, 1910, to the Egyptian Army General Hospital, Abbassia, presented symptoms not encountered in the Khartoum series.

One was admitted with a temperature of 103° F., in a lethargic condition, quite conscious, but with motor aphasia.

The pulse was 116, respirations 28, and some râles and diminution of breath sounds were noted at the right base.

The spleen and liver were not enlarged.

Next day his knee jerks, cremasteric and abdominal reflexes could not be elicited, and there was some cervical rigidity and tenderness over the spine, but Kernig's sign was absent.

He was thereupon isolated, as cases of epidemic cerebro-spinal meningitis were occurring at that time.

Spirochætes found in films

A fair number of spirochætes were found in his blood, and on the third day his temperature rose to 104° F., fell by crisis with profuse sweating, and his speech and reflexes were restored within three days.

He had no further pyrexia, but gave a history of two attacks of fever lasting 6–7 days during the previous month, so that this was probably his second relapse.

The second case was admitted with a temperature of 102° F., slightly enlarged spleen, and considerable pain in both knee-joints.

Blood examination showed nothing abnormal, and, under salicylates, his temperature fell to normal, and he was thought to be suffering from a mild attack of rheumatic fever.

Eight days later his temperature rose to 101° F., and the next day a few spirochætes were found in his blood; the following day very many were found in the films.

On the fifth day his temperature fell by crisis from 103° F. to normal, accompanied by profuse diaphoresis. There were no complications during this attack of fever, but, three days later, his right knee-joint became swollen, hot, very tender, and distended with fluid; there were no constitutional symptoms and no rise of temperature occurred. The condition cleared up in four days without salicylates, and there were no further relapses.

I think the joint condition must have been due to his spirochætal fever, and was not of rheumatic origin; at no time did he have a sore throat.

Morphology of the parasites

Dr. Andrew Balfour has studied the morphology of the spirochætes found in the Khartoum cases (*page* 67), and only a few remarks need be made here.

In some cases the spirochætes were very numerous, in others they could only be found after prolonged search.

They were only found during the periods of fever, and though careful search for possible intra-corpuscular forms during the apyrexial periods was made, yet none were noted.

The long spiral form was by far the commonest, but figures of 8 and coils were frequently seen, though my impression from the dry films was that these latter were far more frequent in the thicker parts of the films, *i.e.* when the spirochætes had more opportunity for movement prior to drying on the slide.

The very excellent arrangement in the Egyptian Military Hospitals of sterilising at once all the clothes of admission cases and then having them washed before being placed in the pack store unfortunately prevented one following up the cases with a view to finding *Pediculi vestimentorum*.

These notes deal with the clinical sides of the cases which supplied Dr. Balfour with the spirochætes for his experimental work.

I wish to thank Dr. Balfour for taking such interest in the cases, and also Mulazim Awal Basili Effendi Suda, Medical Corps, for his help in the clinical and microscopic work.

Temperature Charts

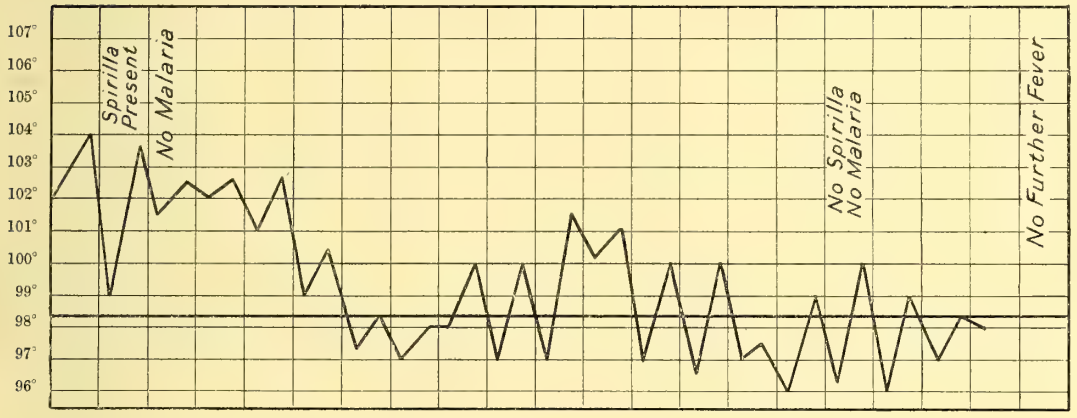


Fig. 18.—Case 2. Anæmia and debility very marked, but no physical signs of any other disease

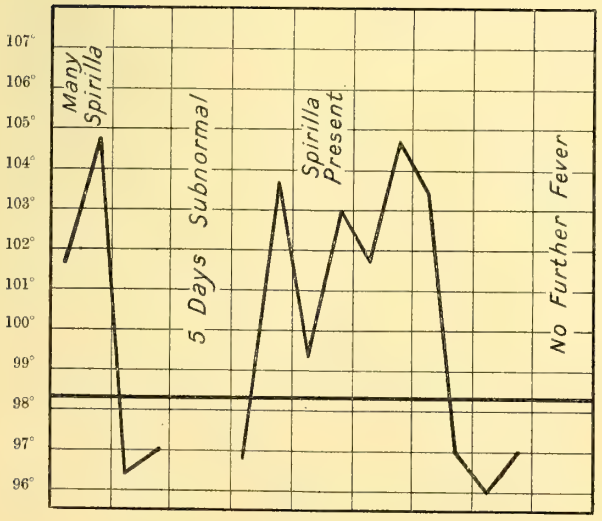


Fig. 19.—Case 3

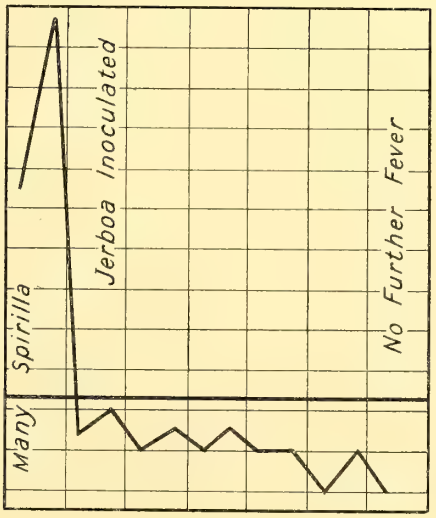


Fig. 20. Case 4

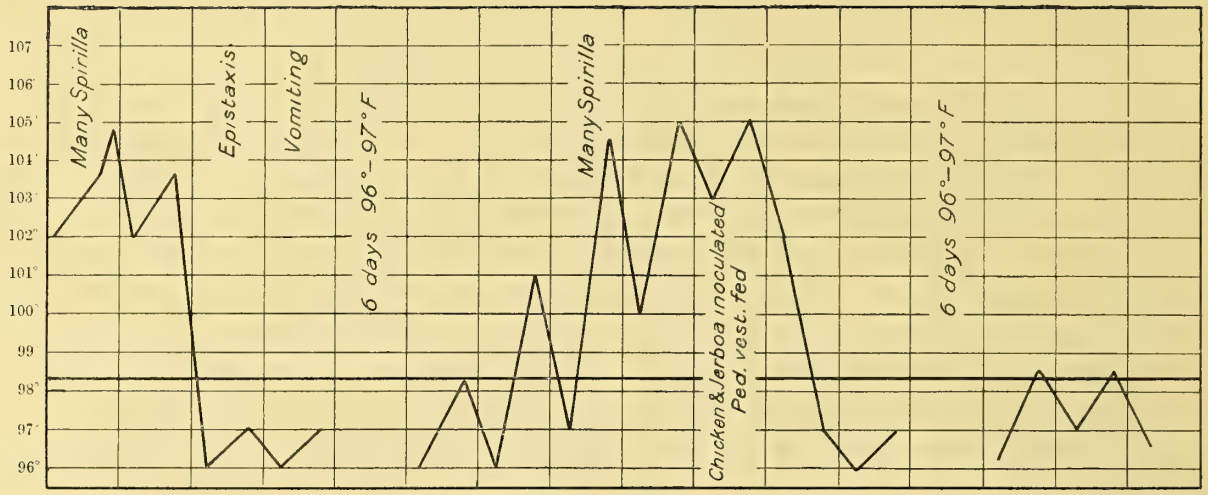


FIG. 21.—CASE 5

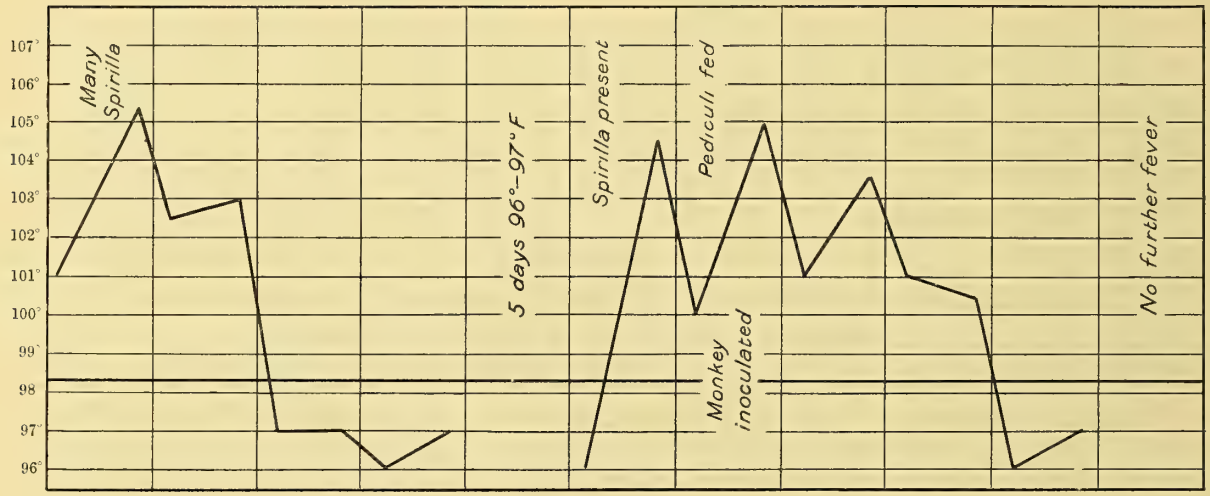


FIG. 22.—CASE 6



FIG. 23.—CASE 7

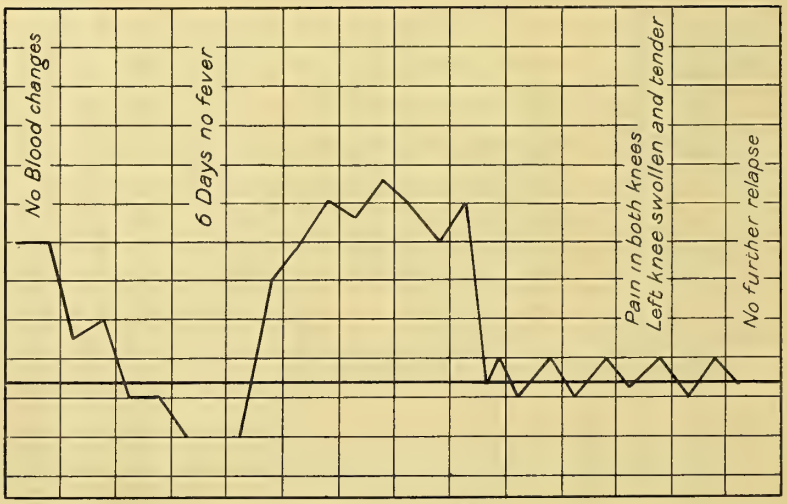


FIG. 24.—CASE 8

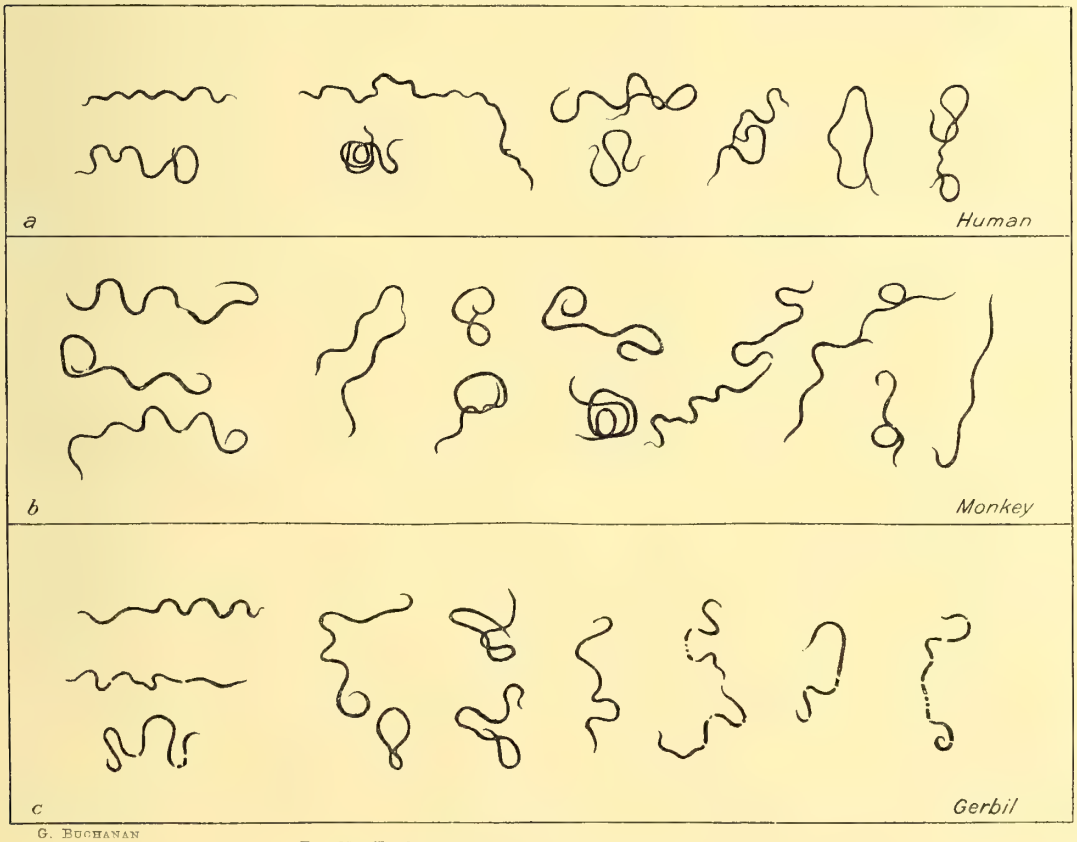
The Spirochæte of Egyptian Relapsing Fever

Is it a Specific Entity?

BY

The Director

Captain Bousfield has asked me to add some notes on the experimental work carried out in connection with the cases of Egyptian spirochætosis he has recorded. I do so with pleasure, but fear they are far from complete. At the time, I was working practically single-handed in the laboratory, being busy with administrative routine and research work, and in addition had my usual duties as Medical Officer of Health for Khartoum to perform. Hence it was impossible to carry out research work on this spirochætal fever in any great detail. The unfortunate loss of the strain, combined with the recovery of all the patients, brought the work to an untimely end, but it has been possible to come to certain fairly definite conclusions which are here stated.



G. Buchanan

Fig. 25.—To show morphological features. Not drawn to scale

Morphology

In the first place a word as to the morphology of the spirochætes concerned. One would like to have studied these by the dark-field illumination method, but at that time I did not possess the necessary apparatus. I only examined stained preparations of human blood, though I observed the parasites in fresh films from the blood of successfully inoculated animals, monkeys and gerbils.

Staining reactions

In films stained by Leishman's method the spirochætes are found to vary in length from 13·5 μ to 22·5 μ, while their breadth is about 0·25 μ. The number of spirals varies from two to six, and the most characteristic feature of the parasites is the great irregularity of these spirals and the marked tendency to the formation of loops, circles and figure-of-8 forms (Fig. 25, *a*, *b*, *c*). A small and narrow spiral is frequently succeeded by a large and wide one, so that there is nothing to be gained by measuring the individual spirals. So far as this feature went there is a distinct resemblance to *Spirochæta duttoni*, and it is noticeable in human, monkey and gerbil blood. For the most part the spirochætes stained uniformly. Where breaks appeared in the ribbon they could usually be explained as divisions between dividing forms. Parasites in gerbil's blood, however, tended to show unstained points in the chromatin core (Fig. 25, *c*). This is also true to a lesser extent of those in monkeys blood (Fig. 25, *b*). In the light of the animal inoculations (*vide infra*), is it to be regarded as an expression of the unsuitability of the medium to the spirochæte? No evidence of longitudinal division has been seen, and never more than two spirochætes have been found united end to end. Their ends are, as a rule, finely pointed.

Animal Inoculations

I. Gerbils

On January 12, 1910, a gerbil or desert mouse (*Gerbillus pygargus*), No. 1, was inoculated subcutaneously with the blood of a patient which showed spirochætes. On January 14, this animal for the first time exhibited parasites in its peripheral blood, an incubation period of two days. The spirochætes were fairly numerous but the animal showed no sign of illness. A few drops of its blood were taken, citrated and inoculated into gerbil 2.

On January 15, spirochætes were still present in the blood of gerbil 1, but they were absent on the 16th, and, despite daily examinations up to the 24th, and occasional observations thereafter, they were never again found. The gerbil at no time appeared ill.

Gerbil 2.—Inoculated from gerbil 1, as stated, on January 14. Showed spirochætes for the first and only time on January 18. Never ill.

Gerbil 3.—Inoculated from gerbil 2, on January 18. Showed spirochætes on January 20, for the first and last time, but it was chloroformed on January 22, as it was looking ill and its coat was rough. Some of its heart's blood was used for the inoculation of a monkey (*vide page* 69).

Gerbil 4.—Inoculated from gerbil 3, on January 20, in order to preserve the strain. A bacterial infection resulted, from which the animal speedily recovered, but, possibly as a result of this accident, it at no time exhibited spirochætes. On January 24 it was chloroformed and a few drops of its heart's blood were inoculated into gerbil 5. No spirochætes could be demonstrated in this heart's blood. Its liver and spleen examined by the Levaditi method also failed to show spirochætes.

Gerbil 5.—Inoculated, as just stated, with apparently sterile heart's blood from gerbil 4, on January 24. It remained perfectly well and at no time exhibited a spirochætal infection.

Gerbil 6.—Inoculated on January 18 with a few drops of citrated finger blood from a patient in the *non-febrile stage*. It never showed spirochætes.

Loss of the strain in gerbils

From the above it will be seen that the strain in gerbils was unfortunately lost.

II. Monkeys (*Cercopithecus sabaeus*)

Monkey No. 1, as already noted, was inoculated with some of the heart's blood of gerbil 3, at a time, however, when the latter animal exhibited no spirochætes in its circulation.

This inoculation proved negative, and, as an opportunity was afforded on January 25 of inoculating this same monkey (the only one which, on that date, could be obtained) with blood from one of the cases in hospital, this was done and the monkey received subcutaneously three drops of finger blood from an Egyptian soldier who had relapsed and was showing spirochætes in his blood. The monkey was found to be infected on January 29, there being a good many spirochætes present in the peripheral circulation. The incubation period was, therefore, apparently four days. This infection increased, and by January 31 might be described as heavy. On this day the animal looked somewhat

Successful inoculation of a monkey

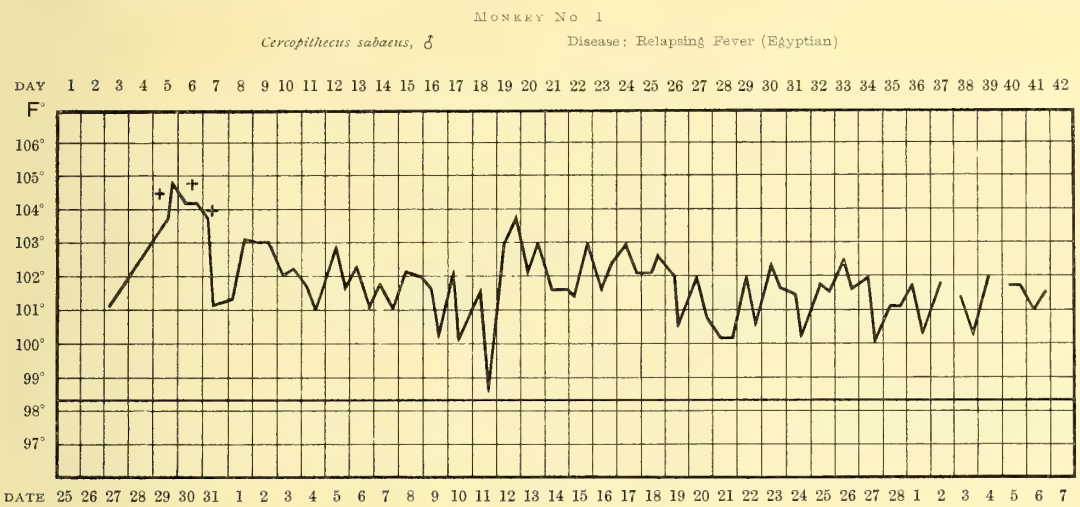


Fig. 26.— + sign = Spirochætes in peripheral circulation

ill. No coryza was noticed. Its temperature was taken daily, and the chart (Fig. 26) shows the course followed. On February 1, though the animal still appeared ill, no spirochætes could be found. On February 2 the monkey looked better and the search was again negative.

With the fear that one had delayed over long, a small quantity of finger blood was taken, citrated and inoculated into monkey 2.

Monkey 1 remained well and did not relapse. Although on February 13 its temperature rose suddenly, no spirochætes were found in its blood.

At the time they were present, lice and ticks were fed on the monkey in connection with experimental work on the possible carrier of the virus (*vide page* 71).

Feeding experiments

Monkey No. 2 (*Cercopithecus sabaeus*).—Inoculated subcutaneously on February 2, from monkey 1, at a time when the latter's temperature was still elevated, although spirochætes had apparently disappeared from its peripheral blood. It is unfortunate that a monkey was not obtainable at an earlier period for inoculation with blood rich in spirochætes, as no infection occurred, but, as will be considered later, this negative result is of some interest and importance. One cannot but regret that great press of work prevented these inoculation experiments being carried out in a more extensive and thorough manner, although it is true that the peculiar behaviour of the strain was one cause of the limiting of the observations. It was only the importance of trying to come to a

conclusion regarding the precise nature of this spirochætosis which induced one to undertake the investigation at a time when one was overburdened with other work which had to be completed. Human spirochætosis being practically unknown in the Sudan, so far as one can tell, it was felt that the opportunity should not be missed.

III. Jerboa

On January 26, a jerboa (*Jaculus gordoni*) was inoculated with finger blood from a relapse case. No infection or illness resulted. This jerboa had a heavy hæmogregarine infection.

IV. Chicken

On January 26, a young chick was inoculated with finger blood from the same relapse case. No infection or illness resulted.

V. Feeding and Insect Transmission Experiments

Experimental work

(1) *Bed-Bugs.*—As stated by Captain Bousfield, a certain number of bed-bugs was secured from crevices in the barrack walls close to where the infected men slept. These were gorged with blood, but dissection of some of them failed to reveal any spirochætes either in their stomach contents or in their tissues. A few were fed on a gerbil and the tissues of several were made into an emulsion and inoculated into another gerbil, but in both instances the result was entirely negative.

(2) *Lice* (*a*).—On January 17, five lice (*Pediculus vestimenti*) were obtained from the clothes of a recruit who had been in contact with the patients. One of these lice was immediately dissected, but no spirochætes were found. The remaining four were placed on a gerbil which was kept in a jar, the base of which stood in a vessel containing water. On January 18, another batch of five lice was secured, on this occasion from the clothes of a patient admitted to hospital three days previously. Two of these lice were dissected and examined, one on January 18, the other on the 19th. In neither case were spirochætes found. The remaining three were placed on the gerbil. The latter, though frequently examined, never showed spirochætes and never appeared ill.

(*b*) An attempt was made to infect lice obtained from the clothes of men at the sewage farm by feeding them on gerbil 2, at the time when its blood contained spirochætes. The experiment failed, all the lice being devoured by the mouse, which was exceedingly active.

(*c*) On January 25, three lice were fed on one of the patients, during a relapse, and at a time when spirochætes were present in his blood. All three became well gorged.

Examination of lice negative

On January 26, two were dissected, one twenty-four hours after feeding, the other thirty hours after feeding. In the first case the whole louse was crushed and examined in the fresh state, then smeared into a film which was stained. In the second case the louse was carefully dissected and the tissues examined in the fresh state only. By an oversight the stained films of the tissues were not investigated.

On January 27, the third louse was dissected and examined piecemeal, both fresh and stained preparations being made.

In none of these lice were any spirochætes observed, despite very prolonged and careful search, nor were any granules observed like those found by Leishman[1] in *Ornithodoros moubata* fed on blood containing *Spirochæta duttoni*, or by myself[2] in *Argas persicus* fed on the spirochætal blood of fowls in the Sudan.

(*d*) On January 27, another batch of nine lice was fed in the morning on a patient showing a fair number of spirochætes in his blood. One of these, a well-gorged insect, was dissected and examined piecemeal with negative results. The remaining eight were fed on monkey 3 (*vide infra*). They were removed from the animal after feeding, and next morning were all found dead, probably owing to the coldness of the night.

(*e*) On January 30, another batch of lice was fed on monkey 1, in the morning, when the animal's temperature was 104·4° F., and spirochætes were present in its blood. Several fed well. A solitary louse was fed in the evening and kept separate from the others, all, however, being maintained at a moist temperature of 37° C. Unfortunately, on January 31, all were found dead.

Indeed, the great difficulty was to keep the lice alive. Three days was the longest period they ever survived, and then only when kept at 37° C. in glass jars containing pieces of paper which had been soaked in human blood. If they died during the night they were usually too dried up in the morning to be of any use for dissection.

Experiment on a monkey

(*f*) *Monkey* No. 3 (*Cercopithecus ruber*).—As already noted under *d*, eight lice which had feasted on a patient were fed on this monkey on January 29. No infection resulted.

Uniform failure, then, marked these lice experiments, and in part this may be due to the small number of insects employed at any one time. They were only intended as preliminary investigations, but, owing to the loss of the strain and the difficulty experienced in keeping the lice alive, are all that one has to present.

(3) *Ticks.*—A few feeding experiments, with subsequent dissection, and, in one instance, the inoculation of emulsified tissues into a monkey, were conducted with *Ornithodoros savignyi*, both nymphs and adults, obtained from Kordofan through the kindness of Captain Cummins.

As the results were entirely negative, they need scarcely be described in detail.

These, then, are the experimental data accumulated, and it will be seen that there is little enough to go on in coming to any conclusion. For all that, there are certain significant findings which may be considered.

Inferences from experiments

One may say at once that, as a result of observing the disease in gerbils and a monkey, and noting the effects of sub-inoculations, one was inclined to regard this fever as possibly due to a specific and hitherto undescribed spirochæte. Captain Bousfield, from his clinical studies, wrote as though it was due to *Spirochæta recurrentis*, but I informed him that I was unable to agree with this view of the case. Shortly afterwards I had an opportunity of showing specimens of spirochætes and giving details of the work to Captain Mackie, I.M.S., who happened to be passing through Khartoum on his way from Uganda. He was inclined to agree with me, but, of course, it was difficult to say anything definite as a result of such meagre and incomplete observations.

It is worthy of note that Dreyer of Cairo,[3] about the time this work was proceeding, published a paper in which he suggested that the spirochæte of Egyptian relapsing fever might be a distinct entity. He was unable, however, to advance any proof in favour of this hypothesis.

Spirochæta berbera

While I was writing this paper, a most interesting account of a form of relapsing fever in South Oran appeared under the joint authorship of Sergent and Foley.[4] They give an excellent account of the symptomatology, etiology and epidemiology of this human spirochætosis, and conclude, on what seem to be good grounds, that this Algerian spirochætal fever is distinct from any form hitherto described, and is caused by a special spirochæte which they propose to name *Spirochæta berbera*, nov. sp.

One would refer the reader to their paper for full details, but it is worthy of note that

	Egyptian *Sp.* (?) possibly *berbera*	Algerian *Sp. berbera* nov. sp.	European *Sp. obermeieri (recurrentis)*	African *Sp. duttoni*	American *Sp. novyi*	Asiatic *Sp. carteri*
Minimal length	13·5 μ, but possibly some coiled forms only 12 μ	12 μ	12 μ	13 μ	7—9 μ ...	12 μ
Shape... ...	Irregular open flexures	Irregular open flexures	Spiral... ...	Open flexures	Regularly spiral	Open flexures.
Flagella ...	?	?	Peritrichous ...	Peritrichous?	Terminal (Novy); Peritrichous (Frænkel)	?
Animals susceptible	Gerbils, but only slightly; monkeys (*Cercopithecus*)	Rats and mice with difficulty; monkeys (*Macacus; Cynocephalus*)	Small rodents only after passage through monkeys	Small rodents and many animals very susceptible	Small rodents very susceptible	Small rodents infected with difficulty.
Course in animals	Very mild	As a rule, mild	Mild	Very severe ...	Severe ...	Very mild.
Sub-inoculations in animals	Gerbil to gerbil positive; monkey to monkey probably negative	Rat to rat or mouse to mouse with difficulty; monkey to monkey negative	Monkey to monkey and mouse to mouse positive (Fülleborn and Meyer)	Monkey to monkey positive; same for most animals (Breinl, Kinghorn and Garrett)	Monkey to monkey and mouse to mouse positive	Monkey to monkey and mouse to mouse positive (Mackie).
Course in man	Fairly severe...	Fairly severe	One, sometimes two, relapses	Severe, four or five relapses	?	Severe, one or two relapses.
Parasites in human blood	Variable ...	Variable ...	Heavy infection	Very sparse ...	?	Variable.
Natural transmission	By lice ? ...	By lice ? ...	?	By ticks ...	?	By lice?
Serum-reaction	?	Immune serum possibly without effect on *Sp. recurrentis* (Russian strain)	Immune serum without any effect on *novyi* and *duttoni*	Immune serum without effect on *novyi* or *obermeieri*	Immune serum without effect on *obermeieri*, *duttoni* or *carteri*	Immune serum without effect on *novyi*

	Egyptian *Sp.* (?) possibly *berbera*	Algerian *Sp. berbera*, nov. sp.	European *Sp. recurrentis*	African *Sp. duttoni*	American *Sp. novyi*	Asiatic *Sp. carteri*
Incubation period in man	Doubtful; possibly more than 12 days	Not stated ...	5—7 days ...	7—10 days.	5—7 days ...	7 days... ...
Duration of first attack	2—8 days ...	5—7 days ...	5—6 „ ...	Average 3 days (rarely 4—5).	5—6 „ ...	5—7 days ...
Duration of apyrexia	2—9 days: 6 apparently the most common	6—16 days: usually 7—8 days	7—10 „ ...	1—8 days (occasionally 10—18).	7—10 „ ...	5—13 days; occasionally up to 19 days
Number of relapses	One or two, possibly three	One or two certainly, possibly others, but very slight	1—2 ...	3—5 (sometimes up to 11).	One (rarely 2—5)	1 relapse in 40 per cent., 2 in 7 per cent. and 3 more in 3 per cent.
Relapses absent	In one case ...	?	?		Not uncommon	In 50 per cent.
Rigors and sweating	Present; rigors only in one case	Rigors not mentioned; sweating marked	Present ...	Rigors in 50 per cent. only, sweating present.	Present ...	Very frequent
Pains in limbs, muscles, etc.	Present ...	Frequent ...	„ ...	Frequent ...	„ ...	„ „
Toxæmia (bilious-typhus type)	Possibly in one case	Absent ...	Mentioned ...	?	Mentioned ...	Present in 10—20 per cent.

	Egyptian *Sp.* (?) possibly *berbera*	Algerian *Sp. berbera*, nov. sp.	European *Sp. recurrentis*	African *Sp. duttoni*	American *Sp. novyi*	Asiatic *Sp. carteri*
Low pulse-rate after crisis	Apparently not noted	No mention ...	Present ...	?	Present ...	Almost invariably present
The tongue ...	White and furred	Moist, white and furred in centre	Large and moist, except in grave infection	?	Large and moist, except in grave infection	Large, flabby and moist, except in grave infection
Appetite ...	Not mentioned: probably therefore never voracious	Not mentioned: probably therefore never voracious	Poor, sometimes voracious	?	Poor	Poor, rarely voracious
Jaundice ...	Absent ...	Exceptional and slight	Mild, except in grave infection	Infrequent in Uganda.	Mild, except in grave infection	Present in 70—80 per cent.; grave in toxæmia
Vomiting of bile	Not mentioned: vomiting present	Not mentioned; vomiting present	Not uncommon	Not usual ...	Not uncommon	Present in 70—80 per cent.
Diarrhœa ...	Absent	Rare	Of brief duration	Always in the Congo; infrequent elsewhere.	Moderate ..	Present in 12 per cent.
Tympanites ...	Not mentioned	Common ...	Grave in toxæmia	?	Grave in toxæmia	Invariably associated with toxæmia
Hiccough ...	,, ,,	Not mentioned	Present ...	Mentioned ...	Present ...	Often present
Hæmorrhage from stomach and intestines	,, ,,	,, ,,	Not frequent...	?	Not frequent	More frequent than in the other varieties
The liver ...	Tender, but not markedly enlarged	Enlarged and tender	Enlarged ...	Enlarged ...	Enlarged ...	Enlarged and tender
The spleen ...	Enlarged and tender	,, ,,	,, ...	,,	,, ...	,, ,,
Parotitis ...	?	?	Mentioned ...	?	? ...	Present in about 10 per cent.
The urine ...	No albuminuria	Dark; excess of urobilin: slight albuminuria	High-coloured, scanty	?	High-coloured	High, bilious, scanty
Hæmaturia ...	Absent ...	Absent ...	?	?	Present ...	? more frequent than other hæmorrhages
Epistaxis ...	Mentioned ...	Mentioned ...	Mentioned ...	Mentioned ...	More frequent than other hæmorrhages	Present in 10—15 per cent.
Pulmonary symptoms	,, ...	,, ...	,, ...	,,	Present ...	Present; more so in toxæmia
Delirium (violent)	Absent ...	Absent ...	,, ...	Infrequent ...	,, ...	Not uncommon; also maniacal
Facial paralysis	,, ...	,, ...	?	Mentioned ...	? ...	Not observed...
Eye affections	,, ...	Slight conjunctival injection	Mentioned ...	Frequent (Moffat, Harrord, and Cook).	Mentioned ...	Present in about 1 per cent.
Herpes labialis	May occur ...	May occur ...	Not uncommon	?	? ...	Not uncommon
Mortality rate	*Nil* (8 cases)...	*Nil* (42 cases)	Very low, under 5 per cent. except in grave infection	13·6 per cent. (?); about 50 per cent. on the Zambesi (?), probably lower.	2 to 4 per cent.; rarely 10 per cent. higher in toxæmia	30 to 40 per cent. in all cases; if toxæmic cases are excluded 15—20 per cent.

while the spirochæte was inoculable from man to the monkey (*Macacus sinensi*, *M. cynomologus*, *M. inuus*, *Cynocephalus sphinx*), it could not be transferred from monkey to monkey.

Now this appears also to be true of the Egyptian spirochæte, although my single inoculation from monkey to monkey (*Cercopithecus sabaeus*), was, most unfortunately, not made at the time most favourable to ensure success.

Sergent and Foley found that rats and mice could be inoculated, but ran a very mild course and recovered. This I found true of gerbils. They also observed that it was well-nigh impossible to obtain successive passages in rats and mice. These sub-inoculations only succeeded in the case of some newly-born mice. I have no parallel observation to record, and had no ordinary laboratory white rats or mice available. I obtained some white rats with all speed from Cairo, but they arrived too late.

Comparison of the Egyptian Spirochætosis with other varieties

Considering the paucity of my experiments and observations, it is perhaps scarcely worth while to enter at great length into a full comparison between this Egyptian spirochætosis, the well-known American, European, African and Indian varieties, and the new Algerian type, but, taking a leaf out of Mackie's[5] and Choksy's[6] books, I have followed the excellent plan of comparative tables adopted by them, and, so far as is possible, have compared all the different species of spirochætes and the fevers they cause. I have added somewhat to Choksy's lists and altered them as seemed desirable, with the results shown in the tables. The entries regarding the symptomatology of the Egyptian fever are, of course, derived from Captain Bousfield's paper, and from such recollection of the cases as I possess.

It is scarcely necessary to point out that such tables as those given must be accepted with some reservation. A great deal, naturally, depends on the personal equation, both as regards the observer and the patient, while environment may play a part. Again, it is scarcely scientific or correct to compare an epidemic of eight cases with one of forty-two, or to compare the symptoms seen in both with those observed in a very large number of cases of the other forms of spirochætosis. Still, bearing these sources of fallacy in mind, the comparisons may be of some service.

This Egyptian spirochætosis is certainly not the true African form due to *Sp. duttoni*; the American variety, for several reasons, can be excluded, and the animal reactions would appear to put the European form out of court. Of the well-known varieties there remains the Indian type, due to *Sp. carteri*. It is strange that Sergent and Foley do not compare their spirochæte with the Indian species, or the Algerian illness with that prevalent in India. This is the more remarkable as there is evidence in both diseases to show that lice act as carriers of the virus, a fact mentioned by the French observers. Perhaps, however, they did not trouble to do so owing to the fact that the results of animal inoculations differ in the two cases. *Sp. carteri* can be transmitted from monkey to monkey and from mouse to mouse. Moreover, the blood of a monkey in the apyrexial stage remains infective, so that Mackie[7] concludes that the spirillum remains in the circulating blood in an unrecognised and ultra-microscopic form.

Probable identity of Egyptian Spirochætosis with that due to *Sp. berbera*

Taking everything into consideration, I believe that Sergent and Foley are justified in regarding the fever in South Oran as a special form due to a specific spirochæte, and, from such facts as one has been able to gather or establish regarding the Egyptian illness, I am inclined to think it is the same disease, due, therefore, to *Sp. berbera*, and conveyed by lice. This, also, despite Strong's[8] note to the effect that he has seen in Egypt infections due both to *Sp. recurrentis* and *Sp. duttoni*.* The view advanced is in favour of Dreyer's supposition, but it is evident that much more work will have to be accomplished before we can be absolutely certain that there is a distinct North African spirochætosis of

man. It is, perhaps, a little surprising that more has not been done in Egypt to clear up this important question. For those desirous of entering more fully into the matter, I append to the attached references a list of recent papers likely to be of use. Some of these are mentioned by Captain Bousfield. Mr. George Buchanan has executed the drawings and prepared the chart with his usual care and skill.

*I wrote Dr. Strong about his cases and he has very kindly replied to the effect that "the case infected apparently by *Sp. duttoni* was observed in a man whom I believe had previously been a sailor on one of the steamers which landed at Port Said. I inoculated white mice with his blood which later showed the parasites. *It seems quite possible that the case might have been an imported one.*" The italics are mine. Dr. Strong does not furnish information regarding the *Sp. recurrentis* cases.—A.B.

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Spirochætosis of Sudanese Fowls

by

The Director

The principal points brought out in the preceding paper on this subject were as follows:—

(*a*) The rôle of *Argas persicus* as a carrier of the disease.

(*b*) The occurrence of an "after phase," or sub-acute and chronic stage, characterised by the presence of curious intra-corpuscular bodies, which were shown to be due, in all probability, to the invasion of the erythrocytes by spirochætes.

(*c*) The breaking up of these bodies, both *in vivo* and *in vitro*, into tiny granules ("spore" forms), which, it was suggested, might play a part in the life-cycle of the parasites.

It seems advisable to give a brief account of the literature on Fowl Spirochætosis which has accumulated since the paper in the Third Report was written.

1. Gareitschnoff[1] in 1907 described a case of fowl spirillosis in Bulgaria, a new locality for the disease which is much more widespread than was originally thought to be the case.
2. Galli-Valerio,[2] in a preliminary note, signalised the production of spirochætosis in fowls at Lausanne by means of ticks (*Argas persicus*) which had been fed on infected fowls in Tunis.

Later[3] he returned to the subject, having carried out numerous experiments. As a result, he came to the conclusion that there is a single form of avian spirochætosis and that therefore the spirochæte with which he worked is *Sp. marchouxi* (*gallinarum*).

Of special interest here is the fact that in a fowl bitten by *A. persicus*, and which succumbed nearly a month afterwards without showing spirochætes, the red cells were found to harbour pyriform bodies which Galli-Valerio thinks resemble the after phase bodies. Spirochætes were not found in sections of the organs stained by the Levaditi-Volpino method. Other forms, even more similar, were discovered in the erythrocytes of a rat inoculated from an infected fowl and which also died. The author is doubtful as to what interpretation to put upon them, but thinks they may be evidence of a feeble infection passing into a chronic state.

3. Comte and Bouquet[4] observed a spirochætal disease of fowls in the oasis of Degache and in the neighbourhood of Tunis, and found that it was transmitted by *A. persicus*. Bats were not susceptible, but geese and ducks could be infected. Brumpt has named the infecting agent *Sp. nicollei* n.sp., as, from its peculiar immunity reaction, he believes it to be a distinct species. It does not protect against itself.

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[4] Comte, C., and Bouquet, H. (1909), "Recherches experimentales sur la spirillose des poules en Tunisie." *Arch. de l'Inst. Past. de Tunis.*, p. 163.

4. Simond, Aubert and Noc[1] described a fowl spirillosis at Martinique which they believed to be identical with that originally found in Brazil. They say it probably exists in Guadeloupe and French Guiana.
5. Mezincescu and Calinescu[2] met with the condition in Roumania where *A. persicus* was common. Ducks and geese were also affected.
6. Brumpt and Foley[3] in 1908 gave a short account of *Sp. gallinarum* in fowls in South Oran.
7. The former author at a later date[4] produced a very interesting paper on a fowl spirochætosis in Senegal. He has named the parasite *Sp. neveuxi* as he found that it could infect fowls which had acquired immunity to *Sp. gallinarum* infection and, conversely, fowls immune to *Sp. neveuxi* contracted the Brazilian septicæmia.

Immunity of adult birds

A point of special importance, and one which from personal experience I believe to be true, is cited by Brumpt. He finds that while chicks are easily inoculated with different species of spirochætes, certain adult birds exhibit a natural immunity conferred apparently by age. Thus he found one very old fowl (five years) which could neither be inoculated by the bites of *Argas* ticks nor by inoculation with *Sp. neveuxi*. He believes this peculiarity to be shared alike by rats as regards the spirochæte of African tick fever (Abyssinian strain). The duck, goose, Java sparrow, sparrow and chaffinch are susceptible to *Sp. neveuxi*. He did not experiment with the pigeon. The infection seemed somewhat less virulent than that produced by *Sp. marchouxi*, but, like the latter, is conveyed by *Argas persicus*.

8. Still later, the same author[5] found that the Somaliland infection was due to *Sp. marchouxi*, or as he calls it *Sp. gallinarum*, and was conveyed, like the other, by *Argas persicus*.
9. Bouet[6] encountered a fowl spirillosis in the French Sudan. By inoculation experiments he apparently proved the identity of this Sudanese spirochæte with *Sp. marchouxi*, and further found it the same as the Senegal virus which Brumpt named *Sp. neveuxi*.

"After phase" bodies found by Bouet

This then would tend to support Galli-Valerio's view. Bouet found my "after phase" bodies in the red cells but would not commit himself to an opinion regarding their nature.

10. Mohn[7] found the disease in the German Cameroons, but of more importance is the work of Blaizot.
11. In 1909, he presented two papers[8 & 9] dealing with the effects of the Somali virus (*Sp. gallinarum*) on chicks of from five days to three months old, comparing the

[1] Simond, Aubert and Noc, F. (April 20, 1909), "Sur l'existence de la spirillose des poules à la Martinique." *C. R. Soc. Biol.*, Vol. 66, p. 714.

[2] Mezincescu, D., and Calinescu, J. (1909), "Spirillose des poules et *Argas persicus* en Roumanie." *Bull. de la Soc. de Path. Exot.*, Vol. 2, p. 292.

[3] Brumpt, E. and Foley (July 18, 1908), "Existence d'une Spirochétose des poules à *Spirochæta gallinarum*, R. Bl., dans le Sud Oranais. Transmission de cette maladie par *Argas persicus*." *Compt. Rend. des Séances de la Soc. de Biol.* Vol. 65, p. 132.

[4] Brumpt, E. (June 9, 1909), "Sur une nouvelle spirochétose des poules du Sénégal produite par *Spirochæta neveuxi* n. sp." *Bull. de la Soc. de Path. Exot.* Vol. 2, p. 285.

[5] Brumpt, E. (July 17, 1909), "Existence d'une Spirochétose des Poules *Spirochæta gallinarum* dans les pays Somali." *Compt. Rend. des Séances de la Soc. de Biol.* Vol. 67, p. 174.

[6] Bouet, G. (June 9, 1909), "Spirillose des Poules au Soudan Français." *Bull. de la Soc. de Path. Exot.* Vol. 2, p. 288.

[7] Mohn (1909), "Über Hühnerspirochätose in Kamerun." Quoted in *Notizen aus der Trop. und Briefk. des Ins. f. Sch. u. Trop. zu Hamb. No.* 5.

[8] Blaizot, L. (October 23, 1909), "Études sur la Spirochétose des Poules produite par *Sp. gallinarum* (Virus Somali). La maladie chez les poussins 1. Modifications de la virulence du parasite par passages directs." *Compt. Rend. des Séances de la Soc. de Biol.* Vol. 67, p. 421.

[9] Blaizot, L. (October 30, 1909), *loc. cit.* Vol. 67, p. 447.

disease in them as produced by tick bite and by inoculation, and contrasting the condition caused by spirochætes taken from birds early in a series of inoculations and those taken late.

The time of obtaining the virus is important

In the case of tick bite the disease was found to be of great severity, the chicks often dying before spirochætes had time to appear in their blood. In the case of inoculation everything depended on the time when the virus was obtained. If early, in the successive passages from chick to chick, a lengthy illness resulted and there was a marked reaction of the organism against the virus; if late, the disease was shorter and there was no reaction.

In a later paper Blaizot,[1] working with the same strain, pointed out the curious fact that while successive passage of the virus through chicks greatly increased its virulence for chicks, it at the same time lessened its virulence for adult fowls.

12. Balfour[2] at an earlier period communicated a paper on further work with the spirochæte of fowls in the Anglo-Egyptian Sudan. As the present article includes and extends these observations they need not be considered here.

13. Prowazek,[3] working at Rio de Janeiro with *Sp. gallinarum*, discovered that this parasite passes through a regular cycle of development in the cœlemic fluid and finally in the salivary glands of *Argas miniatus*.

He figured dividing and resting forms and concluded that the tick is a true host for the spirochæte. His paper is important and requires early confirmation.

14. Dschunkowsky and Luhs[4] at the Veterinary Congress at the Hague, dealt with the spirillosis of geese due to *Sp. anserina*. They managed to cultivate the parasite in a special manner and found atoxyl to be a specific against the disease. Further, they succeeded in preparing a preventive and curative serum from the blood of treated geese.

15. The same authors[5] mention the occurrence of what they consider to be my intra-corpuscular bodies in altered red cells of sick and healthy fowls examined at Elizabethpol and at Surnabad in Transcaucasia. In geese they found two forms, one recalling the bodies, the other in geese suffering from spirillosis and formed of paranuclear bodies included in unchanged red cells.

They ask if the occurrence of these forms in healthy birds does not throw doubt on their parasitic nature, and suggest they may merely be endogenous deformities like Kurloff's bodies or Ferrata's plasmosomes (*vide page* 123 *and* Plate VI., figs. 15 and 3).

One would like to see their specimens before expressing an opinion on the nature of their findings.

Vectors of *S. gallinarum*

16. Schellack[6] has continued the researches mentioned in our Third Report. He finds that *Argas miniatus*, *A. reflexus* and *A. persicus* are all efficient as vectors, while *Ornithodoros moubata* can also transmit *Sp. gallinarum*. *Dermanyssus avium* is not a carrier. The rôle of other ecto-parasites of the fowl as carriers has not been

[1] Blaizot, L. (June 15, 1910), "Études sur la Spirochétose des Poules produite par *Sp. gallinarum* (Virus Somali)—Une propriété de la race cultivée sur poussins." *Compt. Rend des Séances de la Soc. de Biol.* Vol. 68, p. 29.

[2] Balfour, A. (October 1, 1909), "Further observations on Fowl Spirochætosis." *Journal of Tropical Medicine and Hygiene*, Vol. 12, p. 285.

[3] Prowazek, S. (August, 1909), "Contribuiçao para o estudo do dezenvolvimento de '*Spirochæta gallinarum*.'" *Mem. do Inst. Oswaldo Cruz*, Vol. I., No. 2.

[4] Dschunkowsky, E., and Luhs, J. (1909), "Prophylaxis and Pathology of Protozoan Diseases." Quoted in the *Journal of Tropical Medicine and Hygiene*, Vol. XII., p. 292.

[5] Dschunkowsky, E., and Luhs, J. (1910), "Sur l'Étude des Maladies Protozoires des oiseaux domestiques en Transcaucasie." *Rev. gén. de Méd. Vét.*, Vol. XIV., Nos. 163–4.

[6] Schellack, A. (1909), "Versuche zur Übertragung von *Spirochæta gallinarum* und *Spirochæta Obermeieri*." *Arb. aus d. Kaiserl. Gesundheitsamte* Bd. 30 H. 2 s. 352.

demonstrated. If the hen-house is infected, he says that infection *per os* can take place.

17. Tedeschi[1] by the use of abrin killed *Sp. gallinarum*, and thus produced a vaccine which protected canary birds. It is worth noting that he found that *Sp. duttoni* in experimental animals during fever-free periods, remained in the blood as endoglobular forms, both in the erythrocytes and the leucocytes. A protective vaccine

18. Dodd[2] has a paper on fowl spirochætosis in Queensland. He describes the symptoms well, but there is not much that is new in his account. He did not find the "after phase" bodies despite careful search.

19. Blaizot[3] contributed a paper on relapses in the fowl spirochætosis of Tunisia (virus de Degache). He cites the work of Comte and Bouquet, already mentioned, and also the facts as regards recurrence discovered by Bouet and myself. The object of his research was to discover if relapses, characterised by the presence of spirochætes in the blood, occurred in the Tunisian spirochætosis.

This he found to be the case and he points out that so far as we know at present only a heavy, acute infection is capable of conferring a permanent immunity. He points out the close relationship existing in this direction between African fowl spirochætosis and the spirochætal fevers of mammals. He puts forward the ingenious suggestion that both relapses and absence of immunity may be due to a premature crisis occurring before there has been time for saturation with anti-bodies and thereby preventing the first infection being sufficiently severe to ensure a complete immunity. From the study of this question of immunity Blaizot believes that it may yet be possible to affirm that Africa is the original country whence avian spirochætosis spread. His paper is interesting, suggestive and will repay careful perusal.

20. Quite recently, Jowett[4] has discovered fowl spirochætosis in the vicinity of Cape Town. Moreover, along with the disease, he has found intra-corpuscular bodies exactly like those in Sudan fowls. *A. persicus* is the vector, and, judging from the preliminary paper, this spirochætosis of South Africa is identical with our Khartoum form.

21. Gilruth[5] describes the disease from Victoria, in Australia, but did not find endoglobular forms.

22. Dschunkowsky[6] has recently treated the spirillosis of geese successfully with "606," which he finds to be much more active than atoxyl. Successful use of "606"

23. Blaizot[7] in yet another paper returns to the subject and deals with the subsequent cachexia, the question of intracerebral inoculation and the susceptibility of fowls to second infections.

[1] Tedeschi, A. (1910), "Experimenteller Beitrag zur Recurrensfiebers (*Spirochæte Duttoni*)." *Cent. f. Bakt. Orig.*, Abt. I. Bd. 54, H. 1, p. 12.

[2] Dodd, S. (March 31, 1910), "Spirochætosis in Fowls in Queensland." *Journal of Comparative Pathology and Therapeutics*, Vol. XXIII., part 1.

[3] Blaizot, L. (1910), "Note sur la récurrence dans la Spirochætose des Poules en Tunisie (virus de Degache)." *Arch. de l'Inst. Past. de Tunis*, part 2, p. 53.

[4] Jowett, W. (December, 1910), "Fowl Spirochætosis, Note on the Occurrence of, at the Cape."—*The Agricultural Journal of the Cape of Good Hope*, Vol. XXXVII., No. 6.

[5] Gilruth, J. A. (1910), "Note on the existence of Spirochætosis affecting Fowls in Victoria." *Proceedings Royal Society, Victoria*, Vol. XXIII.

[6] Dschunkowsky, E. (January 5, 1911), "Heilversuche mit Ehrlich-Hata "606" bei der Gänsespirillose, etc." *Berl. tierarzth Woch.*

[7] Blaizot, L. (1910), "Nouvelles Recherches sur la Spirochétose des Poules." *Arch. de l'Inst. Past., Tunis*, part IV.

24. Gózony,[1] in a highly interesting paper, shows that spirochætal infection can take place through the unbroken mucous membranes, and believes that the leucocytes play a great part in the production of natural immunity, not, however, by phagocytic action, but by the production of substances injurious to the parasites.

25. Nattan-Larrier[2] deals with hereditary transmission and congenital immunity in spirillosis in several papers worthy of study.

Although the previous work in the laboratories had, as it were, cleared the ground to a considerable extent, certain points still required elucidation, and during the winter of 1908-9, and the spring following, the work was continued as opportunity offered and was also resumed in the succeeding winter with the object of ascertaining:—

Possible relationship of the intra-corpuscular bodies to spirochætes

1. If further proof could be obtained as to the relationship of the intra-corpuscular bodies and the spirochætes.

This necessitated a further study of their morphology, both by the methods previously employed and by such other methods as suggested themselves, notably the employment of dark-field illumination, special staining processes and therapeutic measures, and by a consideration of any analogies which it might be possible to trace in similar or allied hæmatozoa.

2. The course and nature of the disease in chicks. It was found that these were much more convenient for the experimental work than adult birds, but it was soon apparent that conditions in chicks were not analogous to those in fowls. This is interesting as the fact was also noted at a later period by Brumpt and Blaizot in the papers already cited.

3. The fate of spirochætes ingested by *Argas persicus* and the true rôle of this tick as a carrier.

4. If *Ornithodoros savignyi*, the human tick of the Sudan, a species closely allied to *O. moubata*, served also as a vector. Experiments in this direction led me independently to the discovery of the curious bacterial or mycelial-like forms in the tissues of this tick, which Leishman also found, as a matter of fact, in ticks which reached him through Dr. Graham-Smith but which I sent to the latter. They were obtained at a small oasis in the desert near Khartoum. Some time has been spent in trying to determine the nature of these forms.

5. If the fowl lice, so commonly found on the birds, could transmit the disease and, if so, whether or not they were true hosts or merely mechanical vectors.

6. If pigeons and other birds are susceptible to the Khartoum virus.

7. If any other blood changes, besides those already mentioned, required notice.

8. If the Ehrlich-Hata preparation, "606," was effective as a therapeutic agent in this form of spirochætosis.

The great value of the dark-field method

(1) Taking these subjects in the order stated, one found the dark-field method of great service. It confirmed certain observations previously recorded, *i.e.* the entry of spirochætes into red cells and the longitudinal division of the parasites, and enabled these two phenomena to be witnessed with comparative ease and frequency, and latterly it has indicated what is, in all probability, the method by which the bodies are formed. Although spirochætes could be seen entering the red cells and also leaving them, and specimens removed and stained at the proper time showed spirochætes actually in the cytoplasm of the erythroblasts, no formation of bodies directly from spirochætes could be observed.

[1] Gózony, L. (February 13, 1911), "Die Infektionswege und natürliche Immunitat bei Spirochätosen." *Cent. f. Bakt. Orig.*, Vol. LVII., part 6.

[2] Nattan-Larrier, L. (March 10, 1911), "Spirillose héréditaire et immunité congénitale, *et seq.*" *Compt. Rend. Soc. Biol.*

It was found that by this method living and fairly active spirochætes could sometimes be demonstrated thirty hours* after the putting up of the cover-glass preparation, but in no instance, even when they entered corpuscles, did they coil up or break down to form anything approaching the inclusions. These latter are clearly visible by the dark-field method, appearing as silver rings or presenting appearances not unlike tiny cartwheels, owing to their granular contents reflecting the light brilliantly, while their vacuoloid areas remain dark. A "tucking in" of the corpuscular envelope at the point where the body, if peripherally placed, was situated, was often apparent, as, indeed, is the case in stained specimens. The method, however, indicated that the spirochætes eventually break up into granules, and suggested the possibility that it is these granules which really invade the red cells. True, this has never been seen to happen in films of peripheral blood, but blood between a slide and cover-glass is not the same as blood in blood-vessels, and, moreover, granting that these bodies really represent a stage in the spirochæte life-cycle, it is quite likely that they enter the red cells in the internal organs. At first this was merely a supposition. I killed chicks showing good body infections and submitted emulsions of liver, spleen and lung and also the expressed juice from these tissues to dark-field examination, finding a large number of free granules, but it was not until I performed liver puncture and examined the blood thus obtained by the dark-field method (*vide page* 104) that I obtained any definite evidence as to what really seems to happen. Latterly, also, when employing "606" as a therapeutic agent, I have obtained some very suggestive appearances in tissue stained by the Levaditi method (*vide pages* 100 *and* 101).

Breaking up of the spirochætes into "granules"

Acting on Dr. Stevenson's suggestion, I stained films of peripheral blood by the Heidenhain method and counter-stained deeply with saffranin. The result went a long way to show that the bodies have nothing to do with corpuscular or nuclear substance, for, while the red cells stained a dull brownish-grey, their nuclei of course being of a darker hue, the bodies stood out as brilliant pink dots (Plate III., fig. 8), taking on the saffranin stain intensely, but unfortunately diffusely, so that no granular structure could be seen.

It is true that the nuclei of the red cells, possibly because they contain chromatin, exhibited, in some instances, pinkish granules, more especially when the corpuscles stained faintly and were therefore probably degenerated, but these were unlike the bodies, being, as a rule, darker and having irregular outlines. They suggested nodes in the nuclear network. Much the same aspect is presented by films stained by the Heidenhain method and counter-stained with eosin. "Spore" forms of the bodies show black granules when stained by the Levaditi-Yamamato method.

When films were first of all dehæmoglobinised and then stained by either of these two methods the bodies were found to retain the counter-stain for which they possessed an affinity. No further light was, however, thrown on the problem.

Other staining processes did not help one. The silver nitrate method of Khitrovo[1] for *Sp. pallida* was tried as was the Burri Indian ink method, but without any addition to our knowlege being gained. Wet fixation was adopted, but in vain so far as the discovery of anything new as regards the morphology of the bodies was concerned. In this respect one is not further advanced than at the time the Third Report was written, though better specimens of the "spore" forms have been obtained which show the inclusions forming or breaking up into granules and the discharge of the latter

Differential staining

* In one instance I found a still-living spirochæte after 72 hours

[1] Khitrovo (November, 1909), *Archives Méd. Belges*, quoted in *Journal Royal Army Medical Corps*, May, 1910.

from the red cells. (Plate III., fig. 3). In all probability, beyond this "schizogony-like" stage there is nothing more which can be made out.

Experiments in culture

One thought that possibly the use of culture media might help in solving the problem, and, accordingly, blood containing spirochætes or bodies was added to tubes of Novy, MacNeal and Nicolle's media. The spirochætes remained alive and active for 12 days, thereafter becoming sluggish. By the fourteenth day the majority were dead and had lost their staining affinities. In one instance a parasite was found to have entered a red cell. No multiplication was observed.

The intra-corpuscular bodies remained wholly unchanged even when their host cells degenerated.

Little of fresh interest has been made out in preparations stained, as formerly, by the Leishman and Giemsa methods, but Plate III., fig. 7, shows apparently a certain intimate relationship between the inclusions and the spirochætes. This is specially true of the top part in fig. 7 and one of the corpuscles shown under fig. 6. In the former the suggestion afforded is that the spirochætes are entering and coiling up in the red cells. In the latter, the appearance suggests an included spirochæte with a portion of one end still free. I now think these are accidental appearances, but they serve to perplex one, and confuse the issue. Although I have found it impossible to demonstrate the entry of the spirochætes, as such, into the red cells and the subsequent formation of encysted forms exactly like the bodies, I have, as mentioned in the last Report and in this paper, seen spirochætes enter the erythroblasts, a fact which also tended to conceal what I now believe to be the true course of the cycle.

Observations at night

Thinking that the red cell invasion might have something to do with the habits of the tick vectors and therefore might only take place at night I repeated observations previously made at night, but employed, in addition, the dark-field method. Captain Archibald kindly assisted me, but though we worked at midnight and at other hours, no fresh light was thrown on the problem.

Additional evidence that the corpuscular inclusions were in reality stages in the spirochætal life-cycle was, however, obtained by the discovery, on several occasions, that, when the peripheral blood exhibited only bodies, sections of the liver, and sometimes also of the spleen, showed abundant spirochætes. In this connection the following cases may be cited.

1. *Fowl.* White hen. Brought in from market *in extremis* and the peripheral blood on examination found to have a huge intra-corpuscular infection. No spirochætes could be found in the peripheral blood nor were any present in smears made from the lung and bone-marrow in which bodies were numerous. Unfortunately, films were not made from the liver, spleen or kidney, but these organs were stained by the Levaditi-Manouelian method and spirochætes were found in the liver and kidney though not in the spleen and lung. In the kidney sections the red cell infection was apparently also visible. A point of much interest, the significance of which was not recognised at the time, was that many of the spirochætes were fragmented or breaking up into black granules

2. *Chick N.* On *March* 6, 1909, this small, clean, healthy bird was inoculated, subcutaneously, with a few drops of peripheral blood from another chick which showed a very heavy endoglobular infection but no spirochætes.

March 9. A few bodies found for the first time.

March 10. None found to-day, but gaps in the corpuscles noted. These suggested spaces previously occupied by the inclusions.

March 11. A few small bodies again present and some gaps still visible.

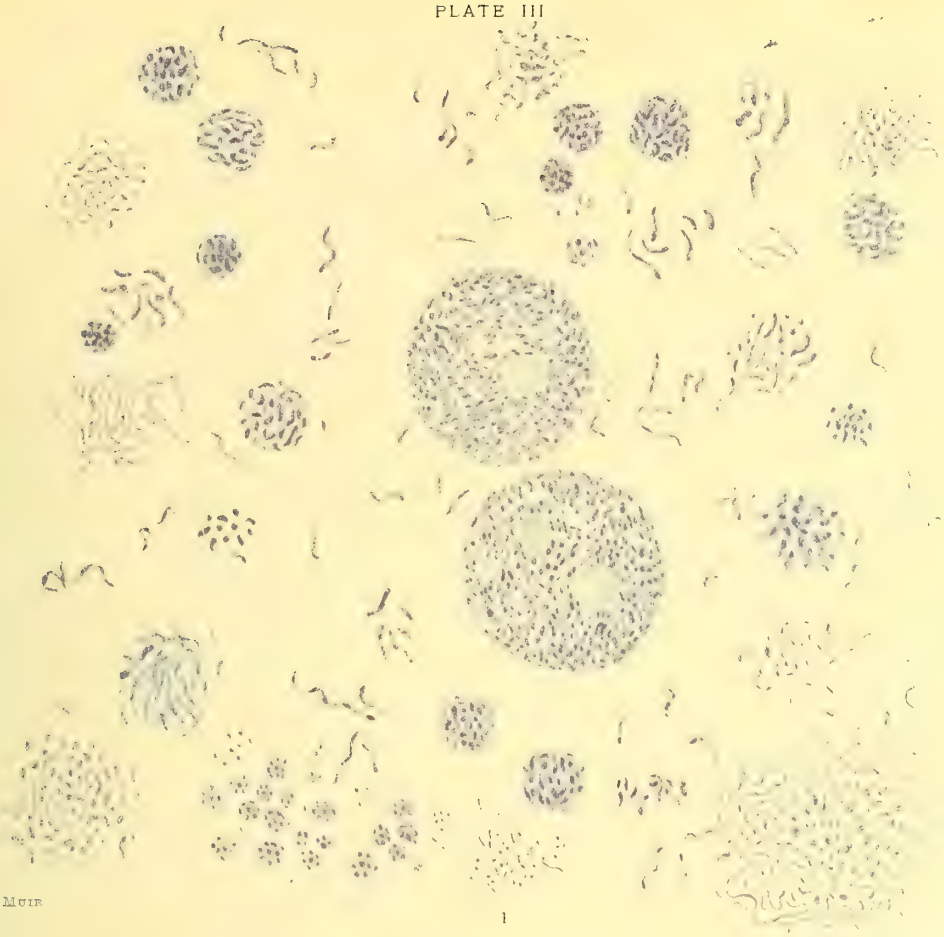
March 17. A great increase in the number of bodies had taken place. "Spore" forms present. Multiple infection of red cells. Bodies of varying size and shape.

Cases

March 18. Literally, a huge infection which presumably caused the death of the bird, though post mortem a greatly distended gall bladder was present.

The red corpuscles of the heart's blood frequently showed many bodies close to their peripheries, there being an apparent gap in the corpuscular envelope where the body impinged against it. This lack of continuity in the corpuscular envelope must not be confounded with the gaps or spaces in the corpuscular substance mentioned above. The liver, examined by the Levaditi-Manouelian method showed undoubted spirochætes lying both in and on the hepatic cells.

PLATE III

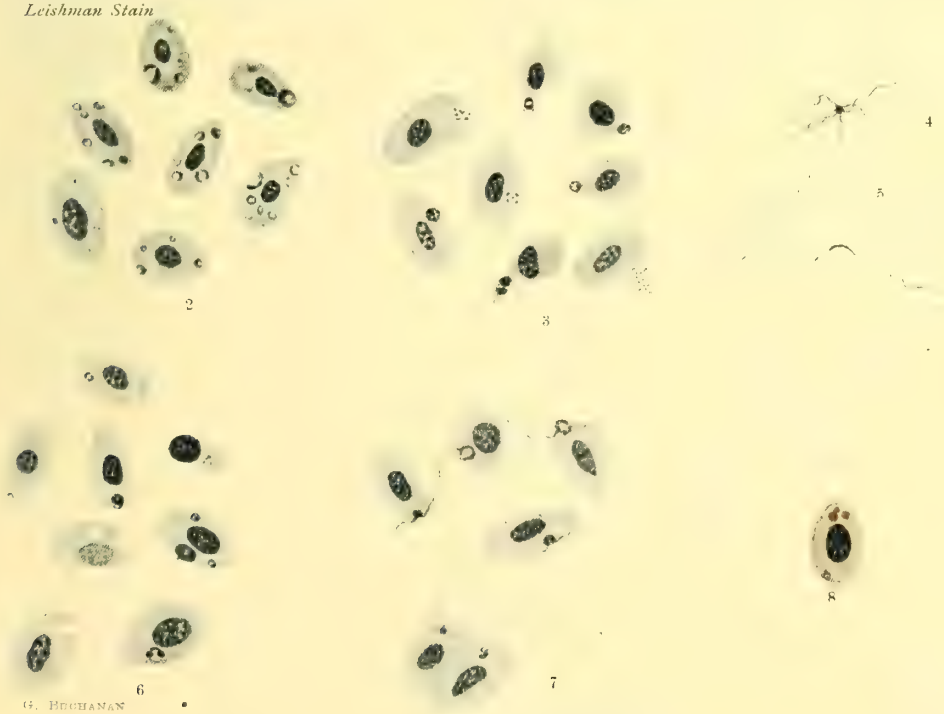


R. D. Muir

1

1. Semi-diagrammatic and composite drawing from smears of portions of one Malpighian tube of a tick (*Argas persicus* ♀) incubated for 23 days at 37° C. after feeding on a chick with acute spirochætosis. Small clumps of Leishman's spirochæte granules and transition of granules into spirochætal forms. The two large nucleated granular cells in the middle are normal tissue cells and are introduced by way of contrast.

Leishman Stain × 1000 *diam.*



2

3

4

5

6

7

8

G. Buchanan

Spirochæta granulosa penetrans, n.sp.

2—8. Cycle of Schizogony

2. Heavy granule infection at different stages
3. Inclusions showing "spore" forms discharging "spores"
4. Clump of spirochætes with central node (peripheral blood)
5. Spirochætes from cœlemic fluid of infected *Argas persicus*
6. Gaps in red cells after discharge of "spores" and inclusion with attached rods
7. Inclusions and free spirochætes in apposition
8. Multiple infection of red cell—special staining

2-7. *Leishman Stain* × 1000 *diam.*

8. *Iron Hæmatoxylin and Seffranin Stain*

Action of "606"

One need not here take up the question of the use of therapeutic agents as a means of settling the above question. The matter will be found discussed under the eighth heading, and it need be merely said that the use of "606" by subcutaneous injection in appropriate doses rapidly drove all spirochætes out of the peripheral blood and appeared also to induce changes in the bodies which led to their eventual disappearance, while, as already mentioned, some highly suggestive results were obtained (*vide pages* 101 *and* 102).

If we search for analogies which may throw light on the subject we find the observations of Galli-Valerio and of Bouet, already quoted, to be at least significant, although neither of these authors is so far prepared to subscribe unreservedly to Sambon's hypothesis or apparently to accept such arguments as one has hitherto adduced. The same may be said of the findings and conclusions of Dschunkowsky and Luhs, though here we are not on such certain ground. For one thing I have only seen extracts of their papers. For another, they seem to be describing several curious appearances in the blood, possibly no one of which is identical with what I have called, possibly erroneously, as will be seen, the "after phase."

It must be remembered also that neither Blaizot nor Dodd were able to find the inclusions, although it is true that they may have been working with a different strain of spirochæte. This is very probably the case so far as the Australian observations go, but the Tunisian strain certainly resembles that with which one has been working in several important particulars. Jowett's recent discoveries in South Africa are naturally of special interest, and his future work may serve to throw confirmatory light on the problem. (See also references to recent papers by Bosanquet and Dobell, *page* 107.)

Another point of interest was kindly brought to my notice by Colonel Sir W. B. Leishman, F.R.S. He told me that he attended a meeting of the Pathological Society of Great Britain and Ireland, where a paper was read by Dr. Henry, on the hæmoprotozoa of British sea-fish. A spirochætosis was described, and at the accompanying lantern demonstration Sir W. B. Leishman noticed inclusions in the red cells of the fish which showed the spirochætes in its blood. He told me these forms closely resembled the bodies of fowl spirochætosis. A portion of Henry's[1] paper has since appeared, but it is only a preliminary note, and though a *Sp. gadi pollachii* has been described as a new species no mention is made of red cell inclusions. A more detailed paper is promised shortly.

Possible analogies to the "granules"

One also finds an account by Tidswell[2] of "rounded bodies," possibly protozoal, in the blood corpuscles of a leather-jacket fish (*Monacanthus sp.*). The description given certainly suggests my "after phase" bodies, but in no instance were more than two found in one red cell. The nature of these bodies is regarded as uncertain. They do not suggest the product of degenerative processes, and the final conclusion reached is that "if they are not protozoal they are more or less normal products of the cell, either when in its younger condition or adult form." They ask if they may be centrosomes like those described by Ross, Moore and Walker in the erythrocyte of the axolotl, the crocodile, man, etc., but find that they are quite unlike these. No statement is made as to whether spirochætes were found in the fish. I should not be surprised to find that fish of this Australian species suffer from spirochætosis.

I mentioned the so-called cytamœbæ of frogs and other reptiles, in the paper on fowl spirochætosis in our Third Report. It is possible that Carini's[3] work on trypanosomes

[1] Henry, Herbert (1910), "On the Hæmoprotozoa of British Sea-Fish (A Preliminary Note)." *Journal of Pathology and Bacteriology*, Vol. XIV., p. 463.

[2] Tidswell, F. (1909), *Reports of the Government Bureau of Microbiology.* New South Wales, p. 45.

[3] Carini, A. (February 25, 1910), "Stades Endoglobulaires des Trypanosomes." *Ann. de l'Inst. Past.*

of frogs throws light on these inclusions and also incidentally on the "after phase." Certainly the spherical chromatin-staining bodies, which he describes and figures as the first stage in the intra-corpuscular life of a trypanosome, closely resemble one form of cytamœba and recall very forcibly the bodies in fowl spirochætosis.

Some analogy may also be traced in the results obtained from the examination of infected ticks (*A. persicus*) presently to be described. As previously noted, the bodies appear to break up into granules which are discharged from the host cells, and certainly these granules recall those found in such abundance in ticks fed on spirochætal blood and first described by Leishman in *Ornithodoros moubata*.

Other somewhat similar conditions may perhaps be found in the changes undergone by the nuclei of trypanosomes in the spleen and possibly in other of the internal organs. The parasites break down and their nuclei disintegrate into small chromatin granules. It is probably from these that the small forms (merozoites?) which invade the red cells in the case of *Schizotrypanum cruzi* and *T. brucei* (*pecaudi*?) of the Sudan (Buchanan) are derived (*vide page* 60).

Again, a recent paper by Gonder[1] on the life-cycle of *Theileria parva* shows, so far as I can follow him, the important part played by the nuclei of the gamonts. These nuclei, however, possess distinct karyosomes, while some are even credited with possessing second nuclei of the nature of blepharoplasts, so that they appear to be more organised than the spirochætal granules. I believe, however, as I stated at the Meeting of the British Medical Association in 1910, that in many protozoal infections the infective "granule" will be found to play an important rôle and to explain conditions which are at present puzzling or obscure. Meyer[2] has reached conclusions very similar to those of Gonder.

Sufficient has been said, both in this paper and in that on Blood Examination (*page* 114), to show that the whole question of red cell inclusions is, with our present knowledge, one of exceptional difficulty. In studying the morphology of these "after phase" bodies I have frequently been puzzled, especially when examining the fresh blood, to account for some of the curious conditions encountered. In the paper to the *Journal of Tropical Medicine and Hygiene* to which reference has already been made (*page* 78), I described certain features which I believed were presented by these bodies in the fresh blood of chicks, and which, though I am now doubtful if the forms on which the observations were made were in reality the bodies of the "after phase," I find it hard to explain in any satisfactory manner. These features were motility and a definite appearance which suggested either a granular structure of the bodies or was evidence that they were possibly coiled-up spirochætes.

The "after phase" bodies

I may very briefly quote from the former paper as regards these two conditions without giving the illustrative details there recorded.

"The spherical, intra-corpuscular bodies in affected chicks are frequently actively motile, circling in one or other direction round the nucleus of the red blood corpuscle. If the corpuscular infection is multiple the bodies may pass each other, may unite or at least lie in close apposition to one another, and, in some cases, may apparently fuse, though it is difficult to speak with certainty as regards this last point.

"The spherical, intra-corpuscular bodies in affected chicks may, whether themselves actively motile or not, exhibit each at its centre what looks like a tiny motile filament

[1] Gonder, R. (December, 1910), "The Life Cycle of Theileria Parva: the Cause of East Coast Fever in Cattle in South Africa." *Journal Comparative Pathology and Therapeutics*, Vol. XXIII. Part 4.

[2] Meyer, K. F. (February 1, 1911), "Beiträge sur Genese und Bedeutung der Kochschen Plasmakugeln in der Pathogenese des Afrikanischen Küstenfiebers." *Cent. für Bakt.* Orig. Vol. LVII. Part 5.

which waves to and fro. The appearance may be roughly imitated by making a ring of the thumb and index of one hand. Then pass the two terminal phalanges of the index finger of the other hand through the centre of the space thus enclosed, and move them so that the tip of the finger waggles briskly to and fro. I have said the appearance is that of a tiny filament, yet this may be an optical illusion, and the curious phenomenon may depend on the active movement of granules into which the body is breaking up. After many observations, however, I am inclined to believe in the presence of an actual filament which may or may not be a portion of a partially encysted spirochæte."

The true "after phase" body is probably not motile

I am now inclined to think that in the blood of these Sudanese fowls two or more "inclusion forms" are to be met with in the red cells which, under certain conditions, may very closely resemble each other. Thus, in the fresh blood, I doubt if the motile bodies described were examples of the true "after phase" forms. These latter were certainly present in the blood, but I am now driven to believe that they are invariably motionless save for the slight oscillations mentioned in the paper in our Third Report. On *page* 49 of that Report it will be seen that one records movement in what appeared to be an "undoubted" body. It did not then occur to me that the blood was being examined under abnormal conditions and that the fact that it was heated probably explained this phenomenon even if the body seen did represent a true "after phase" form, which may not have been the case. Both this body and other motile forms I am now inclined to consider as the vesicles described by Crawley (*page* 118), though it seems to me impossible to distinguish them from the true inclusions which stain well and which I am endeavouring, by advancing further arguments, to prove to be endoglobular stages of the fowl spirochæte in the Anglo-Egyptian Sudan.

As regards the second observation, that of the filament phenomenon, I am still in doubt, not as regards its occurrence, for that has been confirmed by several observers here, but as to its precise significance.

The fact that I have never observed it in the blood of normal gerbils and yet noted it in the blood of several gerbils which had been inoculated, either with human spirochætal blood or the blood of animals successfully inoculated with the spirochætes of Egyptian relapsing fever, would tend to show that it bears some relation to spirochætosis. Some of these gerbils showed spirochætes, for brief periods, in the blood, others did not. In one of the latter, examination of the spleen and liver by the Levaditi method was negative as regards the presence of spirochætes. This, however, in view of the variable results obtained in chicks (*page* 86), means little. That these filament bodies, however, are not the true "after phase" bodies, though exceedingly like them in fresh blood, would seem to be proved by a control case in which a chick with, so far as could be told from examination, perfectly healthy blood, was inoculated with two drops of citrated finger-blood from a case of human spirochætosis (*Sp. berbera ?*). In fresh films on the day following, bodies showing the filament phenomenon were present in or on the red cells, and yet nothing was visible in stained preparations. It is true that careful focussing made me regard these bodies, which were not again found, as epi-corpuscular, but they showed the waving filaments.

Is the condition caused by a toxin?

Is it then possible that, apart altogether from the question of spirochæte invasion of the red cells, there is present in the spirochætal blood some toxic substance which affects the cytoplasm of the erythrocytes producing a peculiar vesicular condition apparent only in the fresh blood and, possibly, in addition, certain degenerative changes which in stained films either resemble or are themselves the true "after phase" bodies? In order

to obtain, if possible, some information on this point the following experiment was carried out:—

January 24, 1911. 5 c.c. of blood drawn with aseptic precautions by a syringe from the wing vein of a fowl showing many spirochætes in its blood. Citrate solution added and the mixture filtered through a small Pasteur-Chamberlain filter F.

Resulting filtrate found clear of parasites and 10 minims of it inoculated subcutaneously into a clean, healthy chick. The result was entirely negative. It is worth noting here that, at a later date, this chick was successfully inoculated with spirochætosis.

Other points relating to the bodies and their connection with the spirochæte infection are so intimately mixed up with the second of our statements that we may at once proceed to its consideration. As space is limited and as records of cases form uninteresting reading it is not proposed to give details of all the experiments carried out (some 70 in number on chicks alone and many more on fowls, obtained both in the open market and from our own specially bred stock), but merely to supply good illustrations as proofs of conclusions stated.

Differences of the disease in chicks and fowls

(2) Reference has already been made (*page* 77) to the fact that there were marked differences between the disease in chicks and that in fowls. The most important are here given in detail, but it must be remembered that to draw definite conclusions from experimental work of this kind requires great care, for, as Levaditi and others have shown, it may be impossible, by ordinary methods, to demonstrate the presence of free spirochætes in the blood of a bird, even though, as shown by inoculation, they actually do exist. At the time most of these experiments were conducted, the use of ricin for facilitating the search, as afterwards advocated by Levaditi and Stanesco,[1] had not been demonstrated and one had to rely on the prolonged search of films which, though important, was very tiring and occupied a great deal of time. For example, if one inoculated a chick with spirochætal blood, and bodies only appeared, and then one inoculated this chick's blood into another bird with the result that spirochætes occurred in the latter, one could never be absolutely certain that there had not been a few spirochætes lurking in the peripheral blood of the first chick, for it would be impossible to examine as a routine the number of films which would represent even the few drops of blood used for an inoculation. Again, it is sometimes very difficult to say whether a bird is absolutely clean or harbours a few bodies.

Bearing these difficulties in mind, the following is the list of the differences to which allusion has been made:—

(*a*) In chicks, not infrequently, the inoculation of blood containing, so far as could be told, free spirochætes only (and these in large numbers) into a clean chick has resulted, not in the appearance of free spirochætes, but of what one regards as the intra-corpuscular stage in that chick's peripheral blood. At a later date, free spirochætes may also appear. The same phenomena may also follow tick-bite or the inoculation of emulsions of the tissues of infected ticks. Hence it would seem that the term "after phase" may, in some respects, be a misnomer. On the other hand, the inoculation of blood, containing apparently only the intra-corpuscular bodies, has now and again resulted in the appearance of free spirochætes at first, and of bodies later. Granting that no spirochætes had been present in the blood used for inoculation, are we to conclude that the spirochætes have been derived from the bodies direct or from infective granules (spores or merozoites) into which the bodies have broken up? Again, supposing that a few spirochætes were present but had been missed, are we to assume that the blood of the inoculated chick was in a condition favouring cell parasitism which consequently occurred, the spirochætes, as granules, at once invading the red cells and appearing as the familiar inclusions?

[1] Levaditi, C., and Stanesco, V. (1910). "Sur un procédé facilitant la recherche des trypanosomes et des filaires dans le sang." *Compt. rend de la Soc. de Biol.*, T. lxvii, pp. 594–596.

I fear I cannot answer these questions, but proceed to give illustrations. Unless otherwise stated, all inoculations were made subcutaneously.

Examples: Experiments

Chicks c and *d*, clean birds, were inoculated on February 18, 1909, from a case of acute, but not very severe, naturally acquired spirochætosis in a fowl. On February 20, chick *c* showed bodies only, and, on the following day, chick *d* followed suit. The bodies increased in number but at no time was the infection in either bird severe. Chick *c*, which showed the most bodies, was chloroformed on March 8 and its liver examined by the Levaditi-Manouelian method but no spirochætes were found. Chick *d* continued to show bodies for a time but they gradually lessened, and, by March 20, had entirely disappeared from the blood. In neither case was there any marked illness.

Chick, clean bird. Inoculated on May 25 with infected tick tissue (*vide infra*). On the following day bodies only found in very considerable numbers (there had been no sign of them in the preliminary examination). On May 29, spirochætes also were found in the blood. An increase had taken place in the number of bodies.

May 30. Increase in the number of spirochætes. Bodies apparently not so numerous as yesterday.

May 31. No bodies found even after prolonged search. Spirochætes fairly numerous. Tangles forming, indicating the crisis.

June 1. No spirochætes. A few bodies.

June 3. As above.

June 5. A few spirochætes and a few bodies found.

June 6. Large numbers of spirochætes. Tangles again forming. No bodies seen.

June 7. As above.

June 8. No spirochætes. Bodies present and showing so-called "spore" forms.

June 9 *to* 13. As above.

June 14. Bird died. Nothing special found post mortem. Section of the liver, spleen and lung were examined by the Levaditi-Volpino method, but no spirochætes were found. Unfortunately the presence or absence of granules was not noted.

This was a most interesting case, and, I am bound to say, suggests a close relationship between the spirochætes and the inclusions. The alternating presence and absence of the latter is, I think, also suggestive in this direction, as is their increase and decrease shown both in this and other cases (*vide infra*).

(*b*) In chicks, relapses are common, and the disease therefore approaches mammalian spirochætosis in type. Spirochætes vanish from the peripheral blood, which may then be quite free or may show bodies. Then, the intra-corpuscular bodies, if present, still persisting, the spirochætes recur. This may happen on several occasions.

Examples:

Chick X. Clean bird. *May* 12, 1909. Inoculated with spirochætal blood from another chick. No bodies present in blood used for inoculation.

May 14. Infected. Spirochætes only.

May 15. Slight increase in number of spirochætes.

May 16. Spirochætes apparently completely absent and remained so till

May 18, when they recurred.

May 20. Spirochætes fairly numerous. Chick ill.

May 21. Found dead. Spirochætes present in heart's blood. Liver shows a few greenish-yellow spots. Microscopically, there was cloudy swelling and the spots were found to be necrotic areas.

Chick V. Bird showing some bodies in the blood prior to inoculation (*vide c* and *d*). Were these true bodies of the "after phase"? Apparently so.

May 8, 1909. Inoculated with blood from chick showing both spirochætes and bodies.

May 9. Both spirochætes and bodies present. Many of the latter very small.

May 10. Enormous number of spirochætes present. Bodies as above. Bird not very ill.

May 11. As above, but tangles numerous to-day. Some of the leucocytes show large, more or less spherical, rose-pink (with Leishman stain) masses in their extra-nuclear portions, the significance of which I am at a loss to understand. Granular white cells, suggesting myelocytes in all but size are present.

May 12. As above.

May 13. Entire absence of spirochætes. Bodies about the same.

May 14. As above.

May 15. Recurrence of spirochætes. Considerable number and tangles. Bodies also increased in number. Multiple infection common. The suggestion is that the spirochætes are now entering the corpuscles, but this was not actually observed. In the fresh blood, motility of what, at the time, were thought to be genuine *bodies* was observed. In stained films, "spore" forms found. One corpuscle seen with a gap in it and, hard by, 15 small chromatin granules observed which may well be spores. (Plate III., fig. 3.)

A few, though each is distinct, are united, the majority are free. Possibly, some of the very tiny bodies in the corpuscles are spores which had re-entered the red cells. This would account for changes in the size of the bodies which may undergo schizogonic development from the merozoite stage.

May 16. Bird found dead. Bacterial invasion. No spirochætes found in internal organs by the Levaditi-Volpino method.

Chick S. This example may also be given as, like V, the bird was inoculated with blood containing both spirochætes and bodies, but eventually recovered.

May 4. Clean bird. Inoculated from another chick.

May 6. Bodies alone found in the blood. It is unfortunate that no examination was made on *May* 5.

May 8. Spirochætes present in large numbers and exist along with the bodies.

May 9. No spirochætes and very few bodies found.

May 10. Only a few small bodies seen.

May 13. As above. Bird very well. Examination discontinued.

Is immunity dependent on age?

(*c*) In chicks, the presence of intra-corpuscular bodies does not signify that the bird is immune to spirochætal infection, as has been found, at least so far, to be the case in adult fowls. Chicks showing bodies have often been successfully inoculated either by tick-bite or the needle, with spirochætal material. Is, therefore, the immunity of the adult bird, as suggested by Brumpt (*page* 77) dependent on age alone? Examples have already been given, but several cases may here be quoted with advantage, not only where spirochætosis developed after inoculation but where inoculation was followed solely, so far as could be told, by a great increase in the number of bodies.

Examples:

Chick bb. Shows bodies in blood.

March 9, 1910. Inoculated from fowl with acute spirochætosis.

March 10. Bodies only present. No change.

March 11. Distinct increase in the number of bodies. No spirochætes.

March 12. Heavy spirochætal infection which persisted along with the bodies till *March* 14, when crisis occurred.

Chick dd. Bird with slight body infection.

June 6, 1910. Placed in cage with batches of ticks (*A. persicus*), some of which, on dissection, showed the infective granules to which reference has been and will be made.

June 7. No change.

June 8. Heavy body infection. Double and multiple infection of red cells not uncommon. This gradually diminished and the bird, which was never really ill, recovered.

Chick ? Its blood contained bodies, it was inoculated with an emulsion of infective tick tissues and developed acute spirochætosis. Details need not be given. The course of the case was similar to that in chick *bb*.

Chick l. Shows bodies in blood.

March 5, 1909. Inoculated from chick, which the day before its blood was taken for this purpose, had shown spirochætes, but to-day (*March* 5), shows only a few bodies. There were, however, in all probability a few free spirochætes still lurking in its blood, though definite proof of this cannot be advanced.

March 6 and 7. No change.

March 10. Very considerable increase in the number of bodies. Multiple infection of red cells. Bird chloroformed. Lung smears showed bodies only. Nothing special noted. Levaditi sections negative so far as presence of spirochætes is concerned.

(*d*) Whatever may be the significance of the bodies it would appear that the spirochæte infection *per se* does not protect against itself. If this is true and if Brumpt is correct, then our spirochæte is like *Sp. nicollei* in this particular. It must be remembered, however, that to arrive at correct conclusions as regards this matter, several points, as Blaizot[1] has quite recently pointed out, require consideration. These are the methods of inoculation, the nature or rather age of the virus as regards passage and the duration of the first attack. One has not had time to work out the question on proper lines, but certain experiments tend to prove the above statement.

Examples:

Chick r. *May* 1, 1909. Inoculated with spirochætal blood from another chick.

May 3. Found heavily infected with spirochætes. Ticks (*A. persicus*) fed on it.

May 4. Both spirochætes and bodies present.

May 5. No spirochætes found to-day. Increase in number of bodies.

May 8. A great further increase in number of bodies. Multiple infection of red cells not uncommon Bird seedy but not very ill.

May 9. As above, but in addition "spore" forms showing to-day when the bird was again inoculated with chick blood showing both bodies and spirochætes.

[1] Blaizot, L. (1910), "Nouvelles Recherches sur la spirochétose des Poules." *Arch. Instit. Past. Tunis.* Pt. IV.

May 10. Bodies as before but a few spirochætes are now present as well. Were these the result of the second inoculation or is it a relapse? It is impossible to say definitely, but in all probability this is a good example of statement *d*.

May 11. Found dead, a fact which rather encourages the view just stated. In Levaditi sections spirochætes were found in the hepatic sinuses.

A chick which had developed spirochætosis from having infected lice placed upon it, which had recovered after a heavy infection and which had shown no spirochætes in its peripheral blood for eight consecutive days received, on January 25, 1911, an injection of fowl's blood containing numerous parasites. It showed no spirochætes up to January 28, on the morning of which day it was found dead. No spirochætes were found in its heart's blood or organs.

A very sudden fall in the temperature of the laboratory during the night may have accounted for death. The case of the chick (*vide page* 86) which received an injection of filtrate from spirochætal blood and yet was subsequently successfully inoculated with spirochætosis, may be taken as another example of *d*, but a better instance is found in the case

(*e*) Very young chicks obtained from the village or market may exhibit a few intra-corpuscular bodies. It is, of course, possible that they may have been infected in the usual way through the agency of ticks, and have passed, either directly or secondarily, into the "after phase," but as there is often no sign of tick infestation upon them, as they usually appear in good health and fair condition, and as the infection is, as a rule, scanty, one is constrained to ask if they may not be examples of hereditary transmissions. It has been shown that *Sp. marchouxi (gallinarum)* can pass into the immature ovum of the fowl, so that it is quite possible that chicks, even live chicks, may be hatched exhibiting a spirochætal infection of this form. It is worth noting that such chicks are not immune, for, if bitten by infected ticks *(A. persicus)* or inoculated with spirochætal blood, free spirochætes appear in their peripheral blood or, as already noted, after a certain inoculation period, the number of intra-corpuscular bodies in their blood undergoes a more or less marked increase. Examples of these statements have been given, but I confess this question of bodies in very young chicks presents difficulties. Are the appearances really the true inclusions, from which they seem to me to be indistinguishable, or do they represent changes in the red cells of the chick or persistence of embryonal conditions? I endeavoured to solve the problem by breeding out chicks from what was, presumably, a healthy stock of fowls, *i.e.* a stock bred on the premises and never infected. In the chicks of a cock and hen of this kind, however, I found corpuscular inclusions, one of these birds being chick *dd* whose case is recorded on *page* 88. It is very difficult, with native attendants who are practically all either thieves or liars, or both, to be sure things are carried out as desired, and with the pressure of work under which one usually labours, it is impossible to keep a close eye on matters of this kind, so that I cannot lay too much stress on these observations which should be repeated. In this country it is hard to obtain fowls which one knows have never had spirochætosis, and I have not latterly been able to obtain foreign breeds which, moreover, are very apt to die.

Possibility of hereditary transmission

It may be a mere coincidence but it seems to me worth recording that the above-mentioned healthy cock and hen of our own stock, when inoculated with infective tick tissues, both showed spirochætes in due course but never showed bodies, though examined at intervals for a month and more. Both birds recovered completely after passing through a transitory leukæmic condition.

(*f*) The injection of blood containing, so far as is known, intra-corpuscular bodies only into chicks may result in an infection of the peripheral blood with bodies only. Such infection, after a time, usually undergoes a great increase, resulting not uncommonly in death, and post mortem spirochætes may be found in the organs.

Example. An excellent example has already been given in the case of chick N (*page* 82) and, curiously enough, a certain chick O, inoculated with a liver emulsion from the bird

used in the inoculation of N (such emulsion containing, so far as could be told, only bodies), ran a course almost precisely similar to N.

Probable increase of virulence in passage from chick to chick

(*g*) Although I have not performed a sufficient number of consecutive transmission experiments on chicks to be quite certain, I believe that the disease increases in virulence when passed from chick to chick. This is what Blaizot found with his virus. I have not observed the effect of such heightened chick virus on fowls.

Section (2) may be suitably concluded by an account of the remarkable history of a certain chick which demonstrated several of the points mentioned, both in this and the preceding section. This account also gives details as to motility and the filament phenomenon previously mentioned (*page* 85).

Chick I, a small, clean chick, was inoculated subcutaneously on *March* 1, 1909, with the heart's blood of a chick which had passed, apparently completely, into the "after phase." The absence of free spirochætes from this latter chick was confirmed by the examination of its liver and spleen, sections of which had been stained by the Levaditi-Manouelian method. No free spirochætes could be seen, but bodies were readily demonstrated in the red blood corpuscles of the blood sinuses.

March 3. Chick exhibited free spirochætes in its peripheral blood in fair numbers. It would appear, therefore, that these must have been derived in some way from their intra-corpuscular bodies in its blood. It is scarcely necessary to add that the chick was protected from tick-bite. (Later knowledge would point to free granules being the source of the spirochætes.)

March 4. No spirochætes found, but bodies had appeared in considerable numbers.

March 6. Bodies more numerous. No spirochætes.

March 7. Bodies still increasing in number. Some large forms noted.

March 8. Condition much the same. Multiple infection of corpuscles common. Bird shows no marked signs of illness, but appears slightly emaciated.

March 9. An apparent decrease in the number of bodies which are, for the most part, of small size. The same note is applicable to the appearance presented on *March* 10.

March 11. A further diminution in the number of bodies was noted. No further examination could be conducted until—

March 17, when it was found that *the free spirochætes had recurred in the blood.* There was a heavy infection, and bodies in small numbers were found to be still present. In one instance a spirochæte was found attached by one end to one of the spherical intra-corpuscular forms. Free spirochætes were also observed, arranged as though radiating from a common centre, a tiny mass or ball of chromatin, and so-called protoplasmic balls, which may be likened to the root nodes of certain plants, were observed on several of the parasites. The bird was carefully examined for lice and ticks, but none were found on it. The blood was pale, watery, and coagulated with great rapidity.

Examination of Fresh Films.—Many active spirochætes. The number of bodies distinctly decreased from that noted in former preparations of fresh blood.

A red corpuscle observed with two spherical bodies in it, one slightly larger than the other. Both bodies were motile, and moved round the corpuscle between its nucleus and its periphery, the movement being, as a rule, from left to right. The waving filament, already mentioned, was seen projecting from the centre of the larger body and lashing to and fro. My assistant, Captain Archibald, and other workers in the laboratories confirmed this observation. The bodies were seen to pass each other, and one apparently disappeared for a time below the other. The corpuscle at this stage underwent change in shape owing to pressure exerted by a neighbouring eosinophile leucocyte, but the bodies remained unaffected for a period of two hours, during which time they were the objects of continued observation. This was then intermitted, and, when last seen, the bodies were motionless, lying side by side, their adjacent "rims" flattened as though they were pressing or being pressed against each other. On the examination of their host corpuscle being renewed two hours later, the bodies could not be recognised, all that was visible being a roughened, granular appearance of the spongioplasm of the corpuscle at the point where the bodies had last been seen. In stained preparations the free spirochætes tended to form loops and rings.

March 18. The condition of motile bodies observed in several corpuscles. One large body showed the waving "filament" at its centre remarkably well. In one corpuscle two motile bodies were seen to undergo what looked exceedingly like a process of fusion. At my request Captain Archibald watched one of the large motile bodies continuously for a period of three hours. He noticed a granule or granules apparently attached to the waving filament which the body presented. The body itself was very actively motile, and, besides movement of translation, kept dancing about between the cell envelope and nucleus. It gradually became indistinct, and the red corpuscle became pale in colour. Finally the body could no longer be detected, but a distinct gap was seen in the corpuscular envelope at the spot opposite which the body had been situated. I looked at the preparation from time to time and confirmed these findings. The fate of the granule and filament remained unknown; they disappeared along with the body. No definite extrusion of the body or of any granules from the corpuscle could be observed.

A stained film led one to think that the number of bodies generally had certainly decreased. The chick remained in very fair health, though somewhat thin; it took food readily.

March 19 and 20. The above notes apply, though, on the latter date, no marked motility was observed in any of the bodies. The blood coagulated with astonishing rapidity, but there was no sign of acute illness. Temperature at mid-day 105° F.

March 21. Some interesting appearances in the fresh blood. Spirochætes fewer. Bodies apparently increased in number. Some corpuscles showed four or even five bodies of different sizes. A corpuscle was

observed exhibiting a large body with four small ones in close attendance, and, in addition, another small body situated by itself. The large body, to which the four small ones had by this time formed a kind of tail, impinged against the small separate body, and was arrested by it. As a result, the "tail" moved on and passed in front of the large body, which itself eventually slipped past the smaller one. In one instance a spirochæte was seen apparently attached, by one end, to a body which formed one of four in a corpuscle; the smallest of the four, the only one not quite spherical in shape, and the only one of the four exhibiting motility. Some spirochætes were still living and motile eight hours after the preparation of this film, which had been kept throughout at room temperature, *i.e.* about 35° C.

March 22. A great reduction in the number of spirochætes both in fresh and in stained films noted. A motile body presenting a filament was watched for one hour. After an interval of two and three-quarter hours the film was again examined, and the body was found to have almost vanished, a localised granular appearance in the spongioplasm of the corpuscle alone remaining. Another body was seen which appeared to be discharging actively motile granules into the *liquor sanguinis*, but I was unable to satisfy myself on this point.

The chick at this date was ill, with ruffled feathers and drooping head, but took food readily.

March 23. Temperature of chick, 105·5° F. No free spirochætes in either fresh or stained films. No increase in the number of intra-corpuscular bodies. Motile forms of these latter again visible, one exhibiting a filament watched both by Captain Archibald and myself. The following is a brief record of the observations:—

5.45 p.m.: Body active and central filament very active, lashing to and fro. 6 p.m.: Only change noted was an appearance as though the body was throwing out a pseudopodium towards the periphery of the red blood corpuscle. 6.15 p.m.: Central filament still active. No further change. 7.20 p.m.: As above.

Observation discontinued, but resumed at 9 p.m. when the body was found to have disappeared, the granular condition of the spongioplasm remaining as in other cases. In addition there was seen a distinct, though slight, protrusion of the corpuscular envelope, opposite the spot where the body had last been seen.

Stained films showed nothing special save that one body exhibited a tiny chromatin rod projecting from it, an appearance previously figured in one of the coloured plates of the Third Report of these laboratories. (Plate VI.)

March 24. No spirochætes, but an undoubted increase in the number of bodies. This was true both of fresh and stained films, but was better marked in the case of the latter. A motile body with filament was observed to contract and change shape prior to becoming practically invisible. Motility was not so marked in the bodies generally, and those showing filaments were distinctly in the minority. In stained films, gaps were seen in some of the corpuscles, and the large size of many of the bodies was noted. Multiple infection was common. The bird was worse and very somnolent, but would wake up to take food, and at times was quite lively.

March 25. No motile bodies and none with filaments seen. Otherwise as above.

March 26. Spirochætes were once more present, though not in large numbers. Several were, to all appearance, attached to the bodies.

March 27. An extraordinary film. The spirochætes were present in enormous numbers, occurring free and in tangles, while the bodies had also undergone a great increase in number. In fresh films they did not exhibit motility or filaments; in stained preparations "spore" forms were visible. The tendency for the free spirochætes to form coils and loops was again noted. By night the chick was very ill and weak, so it was chloroformed and a post mortem performed. There was a condition of general anæmia of the tissues and organs. The heart muscle was exceedingly pale. There were marked green patches on the surface and in the interior of the liver, which proved, on section and staining by Mayer's hæmalum, to be areas of necrosis, the condition being like that sometimes seen in syphilitic livers. The green colour suggested chloroma.

Smears were made from the heart's blood, liver, spleen, lung, and bone-marrow. Those of the lung were most interesting, owing to the vast number of spirochætes present, but otherwise nothing special was noted. The liver, spleen and lung were prepared by the Levaditi-Manouelian method, but no spirochætes could be found in these tissues. Galli-Valerio,[1] using the Volpino-Levaditi method, never found spirochætes in liver sections even when the free parasites were present in the circulation. As will have been noticed, I sometimes succeeded, sometimes failed, but would observe that sometimes a high ocular, Zeiss No. 12, was necessary along with the 1/12th oil immersion in order to make absolutely certain of their presence. Unfortunately the presence or absence of granules was not noted.

In conclusion, I do not know that these earlier researches threw much fresh light on the chief problem—the nature of the bodies. Certain powerful evidence was no doubt forthcoming to show that they are, as previously stated, spirochætal inclusions, but at the same time certain apparently puzzling facts were demonstrated which tended to confuse the issue.

One has not been able to observe their actual formation in the red cells by any of the methods employed and, without this demonstration, any array of proofs is of necessity somewhat incomplete. Still, despite certain difficulties, I adhere, especially in the light of observations yet to be recorded, to my former opinion and, as stated, am forced to believe that these inclusions are not the direct result of spirochætes invading the erythroblasts and breaking down therein, but are due to an entry of small granules derived from the chromatin cores of spirochætes which have already disintegrated. If this be so I am well-nigh persuaded the action takes place in the internal organs, more especially the liver and possibly also in the spleen and lungs. Did it occur

[1] Galli-Valerio *loc. cit.*

in the peripheral blood I think one would have witnessed it by the aid of dark-field illumination, although, of course, the blood is in an abnormal condition and below the temperature found in the sick fowl. (See account of liver puncture case, *page* 102.)

Close connection between the intra-corpuscular bodies and acute spirochætosis

Certain it is that there is an intimate connection between acute spirochætosis of fowls and these inclusions, and also in all probability between the latter and the "infective granules" found in the ticks which serve as vectors in the former.[1]

(3) We now pass to consider the fate of spirochætes ingested by *Argas persicus*, and the true rôle of the tick as a carrier. In some ways this part of the research is the most interesting and important because of the analogies found to exist in this connection between human and comparative pathology. In the first place, however, we may consider a few experiments carried out to see if Prowazek's observations on *Sp. gallinarum* in Brazil *(page* 78*)* could be confirmed in the case of this spirochætosis.

Experiment :

December 5, 1910. A large batch of *A. persicus*, most of them, unfortunately, immature ticks (nymphs), fed on a fowl with a heavy spirochætal infection. No bodies in fowl's blood.

December 8. 3rd day after inoculation. Examination of cœlemic fluid, obtained in the usual way, commenced. A few unaltered spirochætes found. Thereafter a fresh tick was taken on each occasion on which an examination was conducted.

December 9. 4th day. A doubtful dividing form seen.

December 11. 6th day. A fair number of spirochætes found. This suggests some multiplication. Forms suggesting longitudinal division observed.

December 12. Spirochætes present but very few in number. No evidence of division or multiplication. Thereafter examinations on the 14th, 16th and 18th days. All negative.

December 19. 14th day. Two immature ticks examined, one a female. No spirochætes in cœlemic fluid. Following Prowazek's method the salivary glands were dissected out, smeared and examined, also with negative results. There were infective granules *(vide infra)*, in the Malpighian tubes.

Examinations were discontinued on the 16th day after inoculation, nothing further having been found.

In this instance these spirochætes were not found in the ticks after the 7th day. Care was taken to examine ticks which apparently had fed well on the fowl, but it is evident that, unless the observations are very numerous and extended, fallacies may occur.

More work has recently been accomplished in this direction and the above result has to some extent been confirmed. The longest period I have, so far, by ordinary staining methods, found the parasites to persist in the tick is nine days. This occurred in the case of ticks brought me from Jebelin on the White Nile, some 122 miles south of Khartoum where the fowls were ill. These ticks had last fed nine days before examination, and in the Malpighian tube of one of them I found a solitary spirochæte. Examination of the cœlemic fluid proved negative. It would appear, therefore, that there is no such development as Prowazek described for *Sp. gallinarum* in *Argas miniatus* in Brazil. It is interesting to note that the earlier work of Borrel and Marchoux[2] with the same strain and the same tick, but carried out under different conditions, did not yield quite the same results as those obtained by Prowazek. They found that if infected ticks, *Argas miniatus*, were kept at 15-20° C. the spirochætes rapidly disappeared, so that after three to four days none could be found. If, moreover, one then kept the ticks at a temperature of 35° C., even three months later, the parasites speedily reappeared multiplied, and were found especially in the salivary glands. It is possible that this observation derives special interest from the account of a special life-cycle which we now propose to discuss.[3]

Influence of temperature

[1] This and the preceding portion of the paper, suffers from the fact that when they were written, one was working partly in the dark. Still, owing to this very fact, the records of cases possess a certain value and should be considered in the light of results hereafter detailed. There has not been time to re-write the whole article.—A.B.

[2] Borrel, A., and Marchoux, E. (February 25, 1905), "Argas et Spirilles." *C. R. Soc. Biol.* T. 58.

[3] I have since found, by the dark-field method, clumps of motionless sheaths in the contents of the alimentary diverticula of a tick 19 days after it had fed on spirochætal blood. There were no living parasites present. The periplasts stained very faintly with Romanowsky but could be detected in a stained film. There had been no multiplication of spirochætes. The tick had been kept at a temperature of 37° C.

In the second place, therefore, attention is directed to the series of observations conducted on the lines of Leishman's[1] suggestive work on the peculiar chromatin granules which he described as occurring in the tissues of *O. moubata* fed on blood containing *Sp. duttoni*. This work appeared after I had proved that *A. persicus* was a carrier of the Sudan spirochætosis of fowls, and it led me to investigate the matter with a view to finding if the Sudan spirochæte underwent similar changes. Such I speedily found to be the case, and, so far as time permitted, I have endeavoured to follow out Leishman's methods of investigation. It has not, however, been possible to obtain as much leisure as I could have wished. Still the following results have been obtained.

(i) Ticks (*A. persicus*), either as larvæ, nymphs, or adults, fed on chicks with acute spirochætosis, exhibit peculiar chromatin granules.

"Granules" in *A. persicus*

I have found these in the ovaries, eggs, oviducts, alimentary diverticula and salivary glands of the ticks, but more especially and in greatest number in the Malpighian tubes. Sir W. B. Leishman kindly examined my earlier preparations and assured me that the granules, save in some minor particulars, are identical with those found by him.

In carrying out experiments to illustrate this point my difficulty has been to find ticks free of these granules. Spirochætosis is so common and widespread in Khartoum that ticks without these granules in some portion of their tissues are the exception. Again, as Leishman points out both in his first[2] and second[3] papers, "conservative surgery in the case of a tick is unfortunately impossible," and hence "whatever conclusion one arrives at as to the condition of a given batch or brood of ticks, must be founded on the examination of a sample of such batch. However large the sample, and there are obvious limits to this where it is desired to follow up the course of subsequent events in such a batch, any conclusion is open to the objection that a different condition existed in a member of the group which was allowed to survive." It is, however, possible, without killing a tick, to obtain cœlemic fluid by amputating a leg, while the contents of the alimentary diverticula can be obtained by snicking the edge of the body. The tiny wound, as a rule, heals rapidly. I fear I was unable, through sheer lack of time, to make my samples as large as Leishman's, but I did my best, and in this and other work of a like nature I think my controls have been sufficient, for, like Leishman, I have examined a great many ticks in all stages of their development. For the reason above stated it is not easy to say how long a time elapses between the ingestion of spirochætal blood and the appearance of the granules in the place where they are most easily and constantly demonstrated, the Malpighian tubes (Plate IV., fig. 1). We have seen *(page 92)* that the spirochætes themselves do not appear to persist in the tick for a longer period than nine days under ordinary laboratory conditions.

I have found granules within two days of the date of feeding, but cannot be sure that they had not been present before. Until one obtains, by breeding or otherwise, a plentiful supply of granule-free ticks this question cannot be settled.

Tendency of the granules to form "clumps"

As regards the granules themselves, the best idea of their structure and arrangement will be obtained from the coloured drawing (Plate III., fig. 1), where they are shown both in the tissues and lying free. In both situations the tendency to form clumps is very marked. The individual granules are small, usually coccal-shaped, though frequently irregular in outline and, not rarely, appearing like small bacilli. They are so minute that

[1] Leishman, W. B. (February, 1909), "Preliminary Note on Experiments in Connection with the Transmission of Tick Fevers." *Journal of the Royal Army Medical Corps*, Vol. 12, No. 2.

[2] *Loc. cit.*

[3] Leishman, W. B. (January, 1910), "Observations on the Mechanism of Infection in Tick Fever and on the Hereditary Transmission of *Spirochæta Duttoni* in the Tick." *Transactions of the Society of Tropical Medicine and Hygiene*, Vol. 3, No. 3.

it is impossible to make out any definite structure, but it is conceivable that each contains what answers to a nucleus and possibly also some tiny granule corresponding to a blepharoplast. Examination with the highest powers (oil immersion 1/12th, comp. oc. 18, with appropriate tube length) has only enabled one to note that some show points which stain more deeply than their general substance. They take on the chromatin colour strongly and exist in enormous numbers when the infection is at all severe. This is especially true in the Malpighian tubes, in the tissues of which they lie in densely-packed clumps, though the irregular distribution mentioned by Leishman is strongly in evidence. When the tubes are ruptured on the slide the clumps adhere in masses or break up into single agglomerations or into the individual granules of which they are composed. It is then seen that these latter vary greatly in size as well as in shape. In some of the free clumps every individual granule can be clearly seen, others are quite dense and dark, and suggest the presence of a stained matrix holding the granules together. The appearance, however, may merely be due to a somewhat diffuse staining. Like the granules themselves the free clumps vary greatly in size and shape. The smaller ones are often the denser looking. Some are very large and groups of these may occur packed close together, but with the outline of each absolutely distinct, although there is no evidence of any retaining membrane or capsule. The chromatin colour is much better marked in the liberated clumps and granules than in those lying in or on the tissues, for in the latter case the blue background (Leishman or Giemsa) tends to obscure them. It is well, therefore, in every case to lacerate the tissues on the slide before fixing and staining. The granules show up well by the dark-field method, present an active, dancing movement and closely resemble the red cell inclusions of fowls in the earlier stages of the latter.

The granules assume a spirochætal form in ticks kept at 37° C.

(ii) If ticks containing granules are kept in the incubator, at 37° C., in the manner devised by Leishman, the granules may be seen to undergo a change and to assume a spirochætal form. This is confirmatory of Leishman's observations on *Sp. duttoni* in *O. moubata*, though I am not certain that the change is precisely similar or takes place in the same position. Moreover, I have some evidence to show that, at the high summer temperatures prevailing in the Sudan, this change may occur, at least to some extent, without the use of the incubator.

Example :

March 16, 1910. Batch of ticks (*A. persicus*) obtained from a fowl-run and hen-house in Khartoum. Samples examined were found to be heavily infected with granules. These were specially evident in the Malpighian tubes, and it was also found that in this situation the granules had apparently undergone some development, the weather, at the time, being very hot. In addition to granules, short rods, comma forms, and what suggested minute though stout spirochætes, were present.

March 21. Ticks placed in the incubator, at 37° C., along with a dish of water to keep the atmosphere moist.

March 29. One adult ♀ removed from incubator and examined. In the Malpighian tubes there was certainly an appearance which might be construed into a development of young spirochætes from the granules. A few half-developed eggs were removed from the ovary, smeared separately on slides, and stained. Some showed an excellent granule infection but there were no young spirochæte forms.

The alimentary diverticula and contents exhibited no granules or spirochætes. Perhaps this was due to the fact that 13 days, at least, had elapsed since feeding.

Small but numerous granule clumps were seen in a smear of the ovary and two small clumps were observed in that of the salivary glands.

April 4. Adult ♂ examined. Malpighian tubes alone searched. Heavy granule infection and undoubted evidence of development and change into young spirochæte forms.

April 13. Adult ♀ examined. Malpighian tubes showed the above change still more marked. Undoubted spirochæte forms present. The same change, though not so much in evidence, was found in certain parts of the ovary smear.

The oviduct, which contained spermatozoa, and the salivary glands showed no granules.

April 14. Two ticks taken and an emulsion made from their salivary glands. This was inoculated into a healthy cock of our own breeding. Up till April 18 the result was negative. The next examination was made on the 23rd, when the cock was found to be suffering from spirochætosis (*vide page* 95). On the

same day, a healthy hen of our own breeding was inoculated with an emulsion of the ovary and eggs of one of the ticks. The bird developed spirochætosis on the 20th, and ran a mild course. The second tick was a male. No granules were found in its testicles or spermatozoa.

June 11. Adult ♂ dissected, and Malpighian tubes and testicles carefully examined, with entirely negative results.

June 28. Three young males dissected, an emulsion made of their Malpighian tubes, alimentary diverticula and testicles and inoculated into a chick which showed a few intra-corpuscular bodies. Previous examination of the Malpighian tubes had shown the presence of granules.

On July 2 this chick, which was carefully protected from any other source of infection, exhibited spirochætes in its blood (*vide infra*).

The young spirochætes were like those described by Leishman for *Sp. duttoni* in *O. moubata*; shorter, more homogeneous and less regularly curved. I noticed that they took on more of a red than a violet or purple hue when stained by Romanowsky. Indeed, their staining corresponded to that of the granules from which they appeared to develop.

Leishman found his forms apparently actually in the cytoplasm of the cells lining the tubules and the intestinal diverticula. I have not seen any in these situations, but they may have been freed from the cells in the process of making the smears.

(iii) Chicks inoculated with an emulsion of crushed larvæ, showing these granules but no spirochætes, develop acute spirochætosis. The same is true of birds, both chicks and fowls, inoculated with emulsions of washed tissues or eggs from ticks containing these granules. In some instances, in the case of chicks at least, free spirochætes are the first to appear in the blood, followed by bodies later; in other cases the bodies have first of all developed and either persisted unaccompanied or been followed by free spirochætes. It is of special interest to note that whereas a first inoculation of this kind may prove negative, a second inoculation carried out after the material has been kept for several days at 37° C. may prove successful.

Examples. Chick. Very young, clean bird.

May 24, 1909. Inoculated with an emulsion prepared from a batch of 40 young tick larvæ, a few days old. Samples of same batch of larvæ showed granules but no spirochætes.

May 28. Spirochætes found for the first time. (The bird had been kept in a special sterilised cage protected from tick or other insect bite.)

May 29. As above. No increase. No bodies seen.

May 30. Increase of spirochætes. Tangles forming. No bodies observed.

May 31. The crisis has occurred and the blood appears to be wholly free.

June 1. No spirochætes. A few bodies present.

June 3. As above.

June 5. As above.

June 6. Recurrence of spirochætes. Present in large numbers and forming tangles. No bodies seen.

June 7. Spirochætes appear to have again quitted the peripheral blood. Two large films carefully searched. Only a few bodies present.

This blood condition continued till June 14, when the chick was found dead.

Two other good examples (cock and hen, *page* 94) have just been mentioned. In both, the ticks had not fed at all for at least 28 days. Both birds recovered after passing through a transitory leukæmic state. Another and even better example is that of the chick (*page* 95) inoculated successfully with the tissues of three male ticks, for these ticks had not fed at all for no less than 103 days and yet were in an infective condition, the incubation period being five days.

In the cock and hen, intra-corpuscular bodies did not appear, nor were they found in the chick inoculated with an emulsion of larval tissues. They occurred, however, in small numbers in the blood of a clean chick inoculated with an emulsion of tick eggs showing granules, and the two following cases demonstrate their appearance in the blood, in one instance before the spirochætes, in the other, synchronously with them.

Chick. Clean bird.

May 25, 1909. Inoculated with an emulsion of washed Malpighian tube tissue which showed granules but no spirochætes.

May 26. Bodies present in considerable numbers. It seems scarcely possible that they had been missed in the preliminary examination, and yet their rapid appearance is remarkable. They continued present till

May 29, when spirochætes were found for the first time. Some were short, others coiled and in loops. A well-marked increase in the number of bodies was noted.

May 30. Spirochætes more numerous to-day, while the bodies appear to have diminished in number.

May 31. No bodies found even after prolonged search. Spirochætes fairly numerous.

June 1. No spirochætes, a few bodies.

June 3. As above.

June 5. As above.

June 6. Recurrence of spirochætes in large numbers. Tangles present.

June 7. As above.

June 8. Crisis. No spirochætes. Bodies present and many "spore" forms.

June 9 *to* 13. As above. Bodies persisted.
June 14. Died. Nothing to note post mortem.
Chick. Clean bird.
May 25. Inoculated with emulsion of washed and crushed oviduct tissue which showed granules only.
May 29. Spirochætes found for the first time. After a long search a few very small bodies discovered.
May 30. Marked increase both in spirochætes and bodies, and appearances certainly suggestive of invasion of the red cells by the former (Plate III., fig. 7). Some of the bodies are quite large and approximate to "spore" forms.
May 31. Both present but not quite so numerous.
June 1. A few spirochætes but a large number of bodies. Multiple infection of red cells observed.
June 3. No spirochætes. Bodies more numerous and an increase in multiple infection.
June 5. A very heavy infection with bodies. No spirochætes.
June 6. Infection not so great. No spirochætes.
June 7 *to* 10. As above.
June 11. Died.

Significance of the experiments

These cases are highly interesting, not only from the point of view of the infectiveness of tick tissues containing granules, but because they appear to demonstrate, beyond all doubt, a definite relationship between the spirochætes and the erythrocyte inclusions. One has several other records which need not be detailed.

(iv) If batches of ticks, samples of which are found only to show granules in their tissues, be fed on clean chicks, such chicks, after from two to four days, may exhibit, not spirochætes, but the corpuscular inclusions. It is scarcely necessary to give examples, and in any case the observation requires confirmation, as only a few observations were made in this direction. Its possible significance has already been discussed.

The following further points appear to me worthy of consideration and experimental work, but so far I have not been able to pay attention to them. It will be seen that, in part, they are suggested by Leishman's able research.

1. The tracing of the development of the granules in eggs, larvæ and nymphs.

2. An investigation of the excretory fluid of *A. persicus* and inoculation experiments with it. This tick, however, feeds best at night and does not excrete a copious discharge like *Ornithodoros*.

3. The feeding of *uninfected* ticks on chicks showing bodies only in their peripheral blood. As noted it is not easy to obtain ticks wholly devoid of granule infection.

(4) This brings us to the work with *O. savignyi*, the human tick of the Sudan. I do not intend here to enter into the question of the curious rod-shaped bodies which I found in the organs of these ticks, and to which allusion has already been made *(vide page* 80*)*. Captain Mackie, I.M.S., to whom I had the pleasure of showing them, told me he had seen similar appearances in tsetse flies.

Ornithodoros savignyi

One will only consider the relationship of *O. savignyi* to our fowl spirochætosis. I have carried out work with this tick more or less on the same lines as with *A. persicus* and have decided that it is not a true host of the fowl spirochæte. The parasites may be found, either granular or unaltered, for 4 or 5 days in their alimentary diverticulæ, but the special granule infection does not occur as in the fowl tick, at least on a large scale, nor have I found spirochætes elsewhere in the ticks. Moreover, I have never been able to give a chick spirochætosis by allowing *O. savignyi*, which had previously ingested blood from an infected bird, to feed on it, even when the ticks had, in the interval, been kept at 37° C. I have, however, fed *O. savignyi* on chicks showing good "after phase" infections, and by means of them apparently induced a body infection only in a clean chick, or a great increase in the number of bodies in a chick which was not clean but harboured a few of the inclusions. Nothing was found in samples of the tick batches examined, and, unless I mistook some other corpuscular condition for the supposed spirochæte inclusions, it seems difficult to explain these results, unless,

Granule infection, but not spirochætosis, apparently transmitted by *O. savignyi*

indeed, one adopts the view that the few bodies ingested broke up into granules which, though they did not multiply, were yet infective in that they were capable of invading the red cells of any chick into which they were injected, and then undergoing their cycle of schizogony. Perhaps sufficient tests have not been made and a certain proportion of *O. savignyi* may yet be found effective as regards spirochæte transmission. These ticks have to be brought in by camel from an oasis some hours from Khartoum or sent by post from Kordofan so it is not always easy for me to have a good stock in hand. (The above supposition is probably correct as more recent work shows that the spirochætes undergo the same preliminary changes in this tick as they do in *A. persicus*—they shed granules.)

Difficulty in obtaining *O. savignyi*

(5) Can fowl lice (*Menopon* sp. ?) act as vectors? If one could prove these ecto-parasites to be carriers, yet another link would be established in the chain of analogy between this variety of fowl spirochætosis and certain forms of human relapsing fever (Indian and North African). This has now been done. My earlier experiments were either negative or, like the observations recorded in the *Journal of Tropical Medicine and Hygiene* of October 1, 1909, gave only indirect proofs. In this case lice were fed on a chick heavily infected with spirochætes and transferred on March 1, 1909, to a healthy, clean bird, which was carefully protected from tick infestation. Owing to press of other work this chick's blood was not examined till March 5, when its red cells were found to harbour bodies, no spirochætes being found. It was chloroformed and an emulsion of its liver inoculated into a third chick, which, unfortunately, was not a clean bird in that it showed some bodies, but it happened to be the only chick available at the moment. Examination of the liver of the second chick by the Levaditi-Manouelian method proved negative as regards the presence of spirochætes. What happened in the third chick is interesting. On the day (March 6) following the inoculation of liver emulsion a huge increase in the number of bodies took place. On the same date some drops of its peripheral blood were inoculated into a fourth chick; it was chloroformed and an emulsion of its liver injected into a fifth chick. This fourth chick, which is the chick mentioned on *page* 82, developed bodies eventually, died, and its liver was found to contain spirochætes. It may be said that the fifth chick also developed bodies and died, but no spirochætes were found in its liver. The weak link in this chain of events is, of course, the fact that the third chick was not a clean bird. Still, the results were suggestive, and made one think that lice might act as vectors.

Recently, Captain Archibald has carried out some experiments which, I think, definitely confirm this view. Here is one of them:—

Fowl lice are probably vectors

January 10, 1911. Lice removed from a healthy fowl and placed on a chick showing a heavy spirochæte infection. No bodies present.

January 12. Lice removed from the infected chick and placed on a healthy clean chick. Every subsequent day this operation was repeated up to *January* 14. Some twenty lice in all were used. Blood examinations negative till

January 16, when a few spirochætes were found in the blood of the chick on which the successive batches of lice had been placed.

January 17. Heavy spirochætal infection.

This bird eventually recovered without at any time showing intra-corpuscular forms in its peripheral blood.

An experiment of this kind does not of course determine the incubation period after louse-bite, but a subsequent case supplied this information.

January 17, 1911. Two dozen lice from a chick suffering from acute spirochætosis placed on a healthy, clean chick, which was protected from tick bite. Daily blood examinations proved negative until *January* 22, when the bird was found dead with spirochætes in its heart's blood. The incubation period would, therefore, seem to be about four days.

Examination of the lice from this chick proved entirely negative both as regards the presence of spirochætes and of granules, and in this respect resembled all previous examinations of lice. Hence

I am persuaded the lice act merely as mechanical transmitters, and I do not think they play any great part in the spread of the disease. It is true, however, that more dissections are required, for one should not generalise on a comparatively small number of observations.

Pigeons not susceptible

(6) Pigeons do not seem to be susceptible to this form of spirochætosis, as proved by several inoculation experiments. Intra-corpuscular bodies have never been found in the blood of these birds, nor have they ever been found suffering naturally from spirochætosis, although kept in close proximity to infected fowls, and, no doubt, frequently bitten by infective fowl ticks. This tends to show that the Sudan spirochæte is a specific entity or at least that it is not *Sp. marchouxi*. I have not been able to carry out the cross-inoculations advocated by Laveran for the determination of specificity in trypanosomiasis and in any case I doubt the value of this method in spirochætosis. Possibly Levaditi's[1] new "attachment method" may find a use in the latter form of infection.

As mentioned in our Third Report, geese are susceptible to the disease. They suffer from it naturally, and of special interest is the fact that in them the corpuscular inclusions are associated with the spirochætes just as in fowls. Indeed, it was the presence of the former in geese that led me to look for and find the latter, and so prove the condition to be a spirochætal septicæmia.

Dr. Wenyon also found guinea-fowls on the White Nile suffering from spirochætosis, but did not observe any inclusions in their red cells.

I was not able to procure any ducks, and it scarcely seemed necessary to inoculate wild birds on a large scale, as it would have served no useful purpose.

However, a water-wagtail, *Motacilla alba*, was obtained, and received an injection of spirochætal blood on January 25, 1911. Nothing was found in the blood up to January 28, on the morning of which day the bird was found dead. No spirochætal infection found. A small bird, *Passer rufidorsalis*, the local species of house sparrow, also gave a negative result. Here, perhaps, one may mention that further observations on gerbils prove them to be immune to infection.

Blood changes in infected chicks

(7) Blood changes in infected chicks. Some of these were mentioned in our Third Report, and I do not think there is much else to record. In the accompanying severe anæmia the red cells may be greatly changed and, in smears, may be found spindle-shaped and minus their nuclei. As mentioned in the paper on Blood Fallacies *(page* 115*)*, the leucocytes may, as a result of degeneration, show most curious changes. Kurloff's bodies and plasmosomes are sometimes found in them and myelocytes may be present in the blood. Fleeting leukæmic conditions are not uncommon as are increases in the number of the thrombocytes, which are sometimes found in huge masses. These interesting cells may also contain inclusions. The latter may be the "deeply staining (deep purple with Wright's stain) bodies about the size of, or somewhat larger than, a mast cell granule" mentioned by Burnett, and which he believes to be a sign of degeneration. I do not know what they are and have but rarely seen them. The same is true of the large rose-pink bodies found in the leucocytes of chick V. *(page* 87*)*.

(8) *Therapeutic measures.*—In the Sudan, at present, these can only, I fear, have a scientific interest, for the native, who alone keeps and breeds fowls on a large scale, is so careless and apathetic that he would never trouble his head about prevention or treatment. If his fowls die, it is the will of God, if they are ill and recover, to Allah be the praise, and this fatalism is hard to overcome. As an example one may mention what occurred when, in order to try and get the fowl vendors in the Khartoum market to mend their ways and to look after their birds, one offered some substantial (from the native

Treatment

[1] Levaditi, C., and Mutermilch, S. (1910), "Diagnostic des Trypanosomiases par le Phénomène de l'attachment." *Compt. Rend. Soc. Biol.*, Vol. LXIX, No. 38.

point of view) money prizes for the best kept and finest fowls. I was, myself, asked to be the judge, and found, amongst the avian candidates, one hen dying apparently from acute spirochætosis, one cock without a leg, several other deformed birds, not a few infested with vermin, and none that in any civilised country would have been considered for a moment. The only wonder is, that cases of acute enteritis and fowl diphtheria were not likewise present!

I fear, therefore, it will be a long time before the ordinary native takes any real interest in veterinary medicine, and there is, at present, a lack of machinery for educating him in its principles and for pointing out how he might benefit by taking an intelligent and enlightened view of western advance in such matters.

So far then one has been unable to carry out any scheme whereby the serum of infected birds freed from spirochætes, or that of recovered birds, might be tried (possibly in vain) for preventive inoculation or where atoxyl might be employed as a curative remedy. The work with orsudan, mentioned in our Third Report, was brought to an untimely end by the fire in the laboratories and has not been resumed. As I was thinking of testing the effects of atoxyl, which, both as atoxyl itself and as atoxylate of mercury, has been found to be specific for the Brazilian septicæmia of fowls, "606" appeared upon the scientific horizon, and, on reading about its great efficiency in avian spirochætosis, I determined to test it on the Sudan disease and also to see if it would help in clearing up the question of the true nature of the intra-corpuscular bodies.

Experiments with "606"

Professor Ehrlich, to whom I wrote, most kindly and generously sent me a good supply of the drug, and though there has not been time for any extensive series of observations, the following results may be recorded:—

1. Effects on Spirochætes

January 24, 1911. Young fowl weighing 420 grammes sent from the market and found to be suffering from spirochætosis. It was evidently at the beginning of an attack and was not very ill, but showed about one spirochæte in every half-dozen microscopic fields examined (Oc. 1., oil imm. 1/12th Leitz). The bird's skin was hot and it was evidently fevered.

January 25. Infection found to have considerably increased over-night. Spirochætes numerous and forming small tangles. Given ·001 gramme "606" rubbed up with sterile olive oil and injected into the muscles of the thigh.

January 26. Blood found to be quite free from spirochætes. Bird apparently well and fairly lively. Spirochætes never reappeared, but on *January* 30, bodies were found for the first time. The infection was slight. Some were merely tiny granules, others suggested diplococci and others again, though small, were of the usual ring form.

February 1. Increase in number of bodies. Ring forms increasing both in numbers and in size.

February 4. Increase still continuing. Multiple infection of red cells observed.

February 5. Infection now very heavy. Multiple infection common. All stages from the tiny granule to the large "spore" form seen. Small and large forms together in the same corpuscle.

The course of this case suggested that, in addition to freeing the peripheral blood of spirochætes, the drug had broken up these latter in the internal organs into infective granules. These had then invaded the red cells and proceeded to develop in them, finally undergoing a true schizogony with the formation of merozoites.

It would perhaps have been better not to interfere further with the process but merely follow up the case. For better or worse, however, one decided to administer more "606" and on this date about ·002 gramme was given.

February 6. The red cells stain badly but the bodies are very distinct. Though not less numerous they appear shrunken. A few "spore" forms visible.

February 7. Distinct diminution in number of bodies. Some of the red cells have spherical holes in them as if the bodies had fallen out of them. Multiple infection still present.

February 8. No change, so another ·002 gramme "606" given. Weight = 372 grammes.

February 9. A very marked decrease in the number of bodies. No multiple infection seen. The few bodies persisting are small. No gaps or holes seen in red cells.

February 10. Blood very watery and anæmic but bird well. Still further diminution in the number of bodies but a fair number persist.

February 11. No change.

February 12. Possibly a slight increase in the bodies. Weight = 403 grammes.

February 15. As by this date no marked change had occurred the fowl was chloroformed. Weight = 404 grammes. Post mortem nothing special was found. Films of liver, spleen and lung juice examined by the dark-field method but no spirochætes found. None found in stained smears.

Levaditi sections showed some granules in the liver, occurring chiefly in groups and in the capillaries but they were not a marked feature. The lung and spleen exhibited a somewhat similar appearance. In the latter black granules were numerous though I do not think they were derived from broken-down spirochætes. It is not easy to differentiate granular conditions met with in sections stained by the unmodified Levaditi method. No spirochætes were found.

January 25, 1911. Small chick, weighing 80 grammes only, showed on this date the first signs of a spirochætal infection following a laboratory inoculation. A very few spirochætes present in each film of peripheral blood. Given about ·00025 gramme "606" as in former case.

January 26. Blood free from spirochætes. Those in the blood had been driven out of it while increase of the infection had been checked. The bird, however, did not look very well, its feathers being ruffled and its wings drooping.

January 27. Found dying. No spirochætes were found in blood from the heart and lungs. Death may have been due to their sudden destruction or the lethal action of the drug on the chick itself or to the effects of a sudden drop in the temperature of the laboratory.

Liver, spleen and lungs examined by the Levaditi method.

In all the sections, but more especially in the liver, there was found a remarkable appearance of granules closely resembling those found in ticks though, of course, they had not a chromatin colour. They occurred scattered all through the tissues and were evidently not stain deposit.

In order to compare these granules with those found in ticks I stained a portion of the Malpighian tubes of a female *Argas persicus*, heavily infected, by the Levaditi method and found the appearance presented precisely similar to that shown by the chick's liver sections. There can therefore, I think, be little doubt as regards the spirochætal origin of the granules in the latter, and any consideration of their being due to stain deposit or to the peculiar granular condition sometimes found in apparently normal tissues can, I think, be excluded. (Further work in many other cases has amply confirmed this view. The granules found are undoubtedly derived from spirochætes.)

February 20. Fowl. Weight = 741 grammes.

Fair spirochæte infection. Small tangles in the blood. Given ·0025 gramme "606" in oil subcutaneously. A blood film taken 5½ hours afterwards showed a great diminution in the number of spirochætes. Only a few were found after a long search.

February 21. Bird found dying about 7 a.m. No spirochætes in the peripheral blood. Died at 8 a.m. Smear from liver, spleen and lung showed neither spirochætes nor granules, but some suggestive looking granules were seen in liver and spleen juice examined by the dark-field method. It is, however, impossible to distinguish the numerous particles which are seen in such preparations.

Liver sections prepared by the Levaditi method showed granules like those in the small chick which died January 27. In the spleen there were degenerated or necrotic areas which also showed granules though these may not have been of the same nature as those in the liver. Lung sections showed nothing definite. There were no spirochætes.

February 23. Chick. Weight = 62 grammes. Fair spirochæte infection. Given ·0002 gramme "606." The infection had a severe effect, the bird rapidly becoming ill with quickened respirations, drooping head, closed eyes and ruffled feathers. Was this due to sudden liberation of toxines? Six hours after the injection a few spirochætes were still present in a blood film made from a wing vein.

February 24. Found dead, but not yet stiff, in the morning. No spirochætes in heart's blood nor in liver, lung and spleen smears. No granules or corpuscular inclusions seen. Levaditi-Yamamato sections of the internal organs, however, showed the usual general granular condition. In the lung sections there also appeared to be fragments of spirochætes or of periplastic sheaths.[1]

The next case is of considerable interest.

February 23. A fowl which had been brought in from the market exhibiting only bodies was found on this date to be harbouring spirochætes as well in its blood. It weighed 630 grammes and was given ·002 gramme of "606" subcutaneously.

February 24. No spirochætes found in peripheral blood. A distinct diminution in the number of bodies but little, if any, change in those present.

February 25. No spirochætes. Bodies much the same. Given ·003 grammes "606."

February 26. No spirochætes. Increase in the number of bodies and increase in the size of some of them. Bird fairly well, but weak in the legs.

February 27. As above.

February 28. "Spore" forms present to-day. Also multiple infection. Some early granule forms also seen.

March 1. More early granule forms to-day. Not many "spore" forms. Multiple infection still persists.

March 2. Increase in number of bodies. It would seem as though the small granule forms have taken on the ring shape and that the previous ring forms have increased in size and are approaching the schizogony stage.

March 3. More large "spore" forms to-day. Some with "exit gaps." Small ring forms also present.

March 4. No change noted from yesterday.

March 5. Found dead. Heart's blood as above. No spirochætes found in liver juice by the dark-field method.

An examination of sections of liver, spleen and lung stained by the Levaditi method shows throughout a fine granulation which suggests a spirochætal origin. No spirochætes found.

[1] In a later very interesting case, not only was a typical granule infection present, but in liver sections spirochætes, or rather spirochætal sheaths, could be seen with similar black granules in them.

There is, I think, no need to multiply the records of cases; "606" in sufficient doses can certainly banish spirochætes from the peripheral blood and in certain instances absolutely cure the bird. In other cases the granule phase supervenes on which the drug has apparently only a limited effect and sometimes, possibly, none at all. This question will be discussed immediately, and the nature and significance of the action of "606" will be found dealt with in that part of the paper termed "The Mechanism of the Crisis" (*vide page* 102). Results are favourable

It only remains to be said that chicks are very susceptible to the toxic effects of the drug, and that it is much more easy to cure adult fowls with it than the delicate and immature bird which usually perishes from the combined effects of the infection and the medication.

2. Effects on Intra-corpuscular Forms

In one of the earlier experiments, a chick with a heavy "body" infection was given too large a dose of "606" and died a few hours afterwards. Apparently as a result of the administration of the drug, while there was no diminution in the number of bodies found in the heart's blood, they had undergone considerable change, breaking up into "spore" forms, several of which were discharging granules from the red cells. These granules were found lying free in clusters (Plate IV., fig. 3)*.

It is perhaps scarcely necessary to give in detail the dozen experiments carried out to discover how "606" affects the inclusions. It may be said at once, that, as was to be expected, there is no rapid action on them as upon the spirochætes. In the majority of cases, however, the drug seems to hasten the disappearance of the bodies. It is, of course, difficult to say in any individual case that the disappearance is due either wholly or in part to the "606" as the condition tends towards cure in many cases.

Still, when one finds a fowl with a heavy infection treated by the drug and its blood freed permanently from the intra-corpuscular forms in the short space of eight days, the result is suggestive. In some instances, as in the case recorded above, "606" appears to hasten the breaking up of the bodies into "spores" and the discharge of the latter from the red cells. In such cases I have seen the infection increase, possibly because some of the free granules escaped destruction and re-invaded the erythroblasts. There is no doubt that they are much more resistant than the free spirochætes and this fact may yet throw some light on the persistence of spirochætal infections in man. Death frequently results in the case of fowls in the "after phase," or as one may now perhaps call it the "granule phase," when they are treated by the Ehrlich-Hata preparation. The granules are more resistant than free spirochætes

It will be necessary to carry out many more experiments before the precise action of the drug on the inclusions and the infected birds can be ascertained.

In some of the fatal cases the organs examined by the Levaditi method, and more especially the liver and lung, showed rather suggestive clumps of little black granules which may have resulted from the blood infection, but I am not prepared to be dogmatic as to their origin. On no occasion was any appearance met with resembling the great granule infection presented by the organs of the chick treated for acute spirochætosis by "606."

The Mechanism of the Crisis

As Revealed by the Dark-Field Method

I now pass to some most interesting and suggestive work recently carried out and still uncompleted, which, as already indicated in several places throughout this paper,

* *For* Plate IV., fig. 3 *read* Plate III., fig. 3

has, I believe, cleared up the problem of this fowl spirochætosis in a satisfactory manner. It will be apparent that "606" produces what may be called an artificial crisis, and this gave one an opportunity of discovering, more readily than one can do under natural conditions, what happens to the spirochætes after the peripheral blood has been freed or nearly freed of them. For this purpose liver puncture was employed, and at a later date well-infected chicks which had been given a dose of "606" and whose peripheral blood no longer harboured parasites or showed only very few of them were chloroformed, and blood was quickly taken by means of aspiration from the liver, spleen and lung. If then a drop of liver juice be examined by the dark-field method it will be found swarming with spirochætes and with highly refractile granules. The source of the latter is soon apparent, for attention will be directed to spirochætes which are not moving in the usual way but are in a state of violent contortion or are, so to speak, shaking themselves to and fro. Indeed I cannot give a more apt comparison than by likening their movements to those of dogs which have been in water and are shaking themselves vigorously to dry their coats. The object of the spirochætes, however, is to rid themselves of the bright, spherical granules which can be seen within them and which may or may not be aggregations of the so-called chromatin core (Plate IV., fig. 2). These are forced along the periplastic sheath or cell membrane and suddenly discharged from one or other end of the parasite, so that they become free in the medium and dance hither and thither as tiny, solid, spherical, brilliantly white particles. In process of time the spirochæte loses its activity, becomes difficult to see and eventually all that is left of it is the limp and lifeless sheath drifting aimlessly in the fluid and liable to be caught up and swept away by some still vigorous parasite. Such a sheath may still retain one or two of the granules which it has been unable to discharge. As may be imagined the process is most fascinating to watch and my observations have been confirmed by Captain Fry and Mr. Buchanan of these laboratories and by Major Ensor and Captain O'Farrel, R.A.M.C. I may also say that the first named had previously seen a shedding-off of granules by trypanosomes in the peripheral blood of experimental animals, a phenomenon which he is now studying.

Artificial crisis

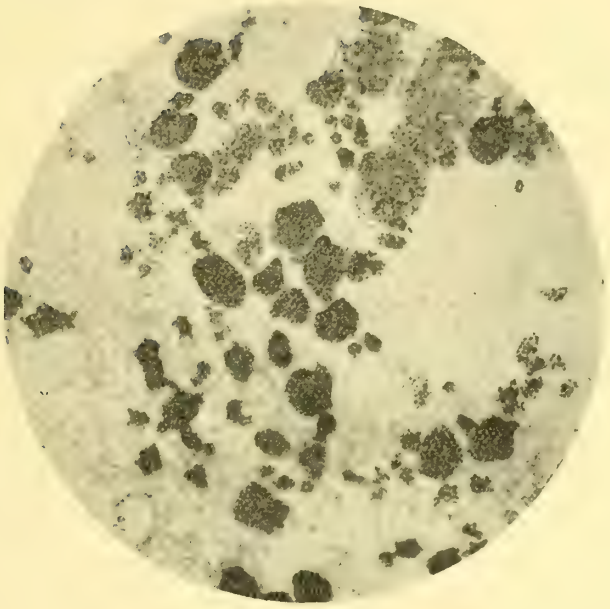
Granules shed from the spirochætes

The same phenomenon is to be witnessed in films from the spleen and lung and may yet be found to occur, under certain conditions, in the peripheral blood.[1] All the spirochætes seen do not exhibit this granule shedding. Some will be found dead and motionless, killed outright by the drug. Some look and move like ordinary parasites but it is probable that in the long run these start the convulsive movements and discharge granules, as if a film be examined after 24 hours it is customary to find no living spirochætes, only a few "shadow" sheaths with or without a few bright granules in them. The spirochætes which exhibit these remarkable changes are easily distinguished by their peculiar movements. These are not continuous. Between the convulsive shakes the spirochætes straighten themselves and lie motionless as if exhausted, then all of a sudden they are again thrown into violent contortions during which very often the granules can be seen shifting to and fro within the sheath. As a rule, however, they are driven towards one end and then by means of a final flicker set free. As a rule, after expulsion of a granule, quiescence occurs, but this is not always the case. Observation has to be both close and unremitting, as even in a very thin and small film numerous free granules are usually already present and if these are in the

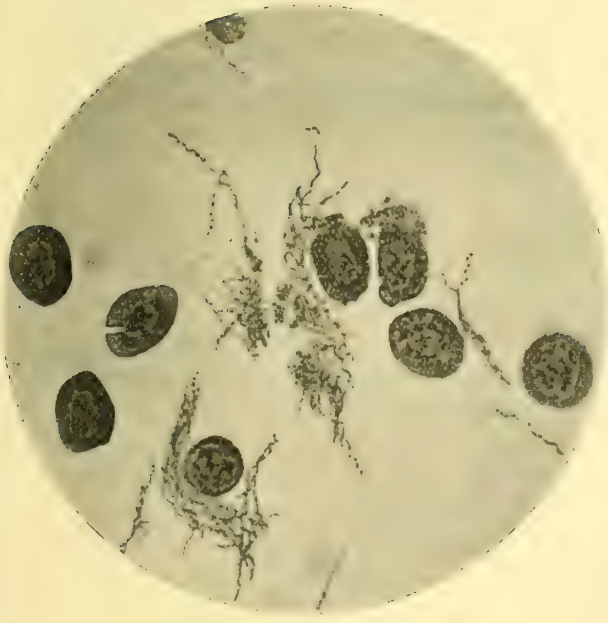
Changes in the spirochætes during shedding of granules

[1] Is it possible that the coccoid bodies described in the blood of relapsing fever cases are, in reality, these spirochætal granules? I have now found that the granule shedding does occur in the peripheral blood under certain conditions.

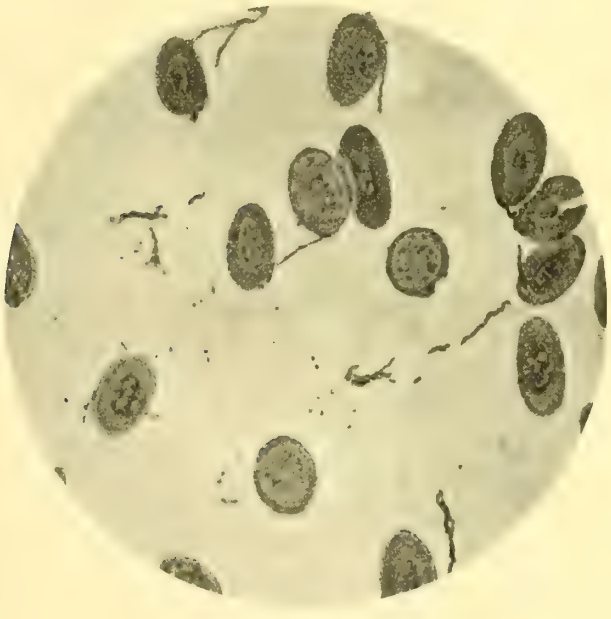
PLATE IV



1



2



3

G. Buchanan

Spirochætosis of Sudanese Fowls

1. Malpighian tube of *Argas persicus* showing granule infection
2. Chick's blood showing granules in spirochætes
3. Chick's blood showing free granules

× 1000 *diam.*

neighbourhood of the discharging spirochæte it is often very difficult to say if a granule, suddenly appearing, has actually just been ejected from the parasite, the more so as the latter tends to lash and shake itself out of focus.

After the process has gone on for some time the whole spirochæte appears shorter and broader, more ribbon-like in fact, and this would appear to be due to a process of flattening owing to loss of its central substance. It should be noted that under the conditions mentioned the red cells are fragile and will often be found undergoing curious changes giving rise to what I have termed the chain, droplet, dumb-bell and filament phenomenon (*page* 115 and Fig. 30) first described by Nuttall and Graham-Smith. The curious slender motile filaments might easily be mistaken by the inexperienced for altered spirochætes though the droplets or rings into which they disintegrate, owing to their low refractile indices, could scarcely be taken for the infective granules. It is highly probable that these granules are derived from the chromatic core as, after their discharge, nothing but a sheath which stains but faintly with Romanowsky remains unless, indeed, the shedding has not been quite complete when granules are still to be seen inside the sheath (Plate IV., fig. 3). At the same time it is a remarkable fact that the free granules themselves do not appear to take on this stain. Only on one occasion have I found them faintly indicated. They might easily have been missed. They are, however, readily coloured by the Levaditi-Yamamato method and then appear as black, homogeneous, spherical dots scattered in great profusion throughout the smear. It is possible that these granules possess an affinity to those recently described by Dobell[1] in *Cristispira veneris*. As noted, the granules on being set free are very active. They dance hither and thither and sometimes appear to increase in size. Beyond this change I have been unable to discover what happens to them. I have never seen them enter red cells in preparations of the kind mentioned and they appear eventually to die in these cover-glass films. At the same time they require further watching in *cases where the crisis occurs naturally and in which, be it noted, precisely the same phenomenon occurs although no drug has been administered.*

Changes in the red cells

So far I have not seen it in the peripheral blood, even in cases treated by "606," but my observations have been limited and unsatisfactory.* In the case of a chick which had passed the crisis *naturally* on the previous day and whose blood was free of spirochætes but was beginning to exhibit bodies, I witnessed what appeared to be the entry of a free granule into a red cell. This was in a thin liver juice film examined by the dark-field method and in which were many free granules and a few red cells containing bodies. Certainly a bright granule invaginated the cell envelope and apparently made its way into the cytoplasm. It then became partly surrounded either by a portion of the invaginated cell envelope or by a capsule of its own formation. As one was watching the process with deep interest a sudden current in the film carried the corpuscle away nor could it again be found. Major Ensor and Captain Fry, who were interested spectators of this process, were both inclined to regard it as an entry of one of the granules into a red cell. Certainly there can be no doubt as to the invagination, but it is evident that more observations are required. (These have now been made on peripheral blood, liver, spleen and lung juice and bone-marrow preparations, but as yet in vain.)

Do the granules enter the red cells?

[1] Dobell, C.C. (April, 1911), "On *Cristispira veneris* nov. sp., and the affinities and Classification of Spirochætes." *Quarterly Journal Microscopical Science.*

* Since writing the above I have witnessed spirochætes shedding granules exactly as described in a film of blood from the heart of a very young chick which had been inoculated 33 hours previously with blood from a small chick just before the crisis, and which developed both spirochætes and bodies almost simultaneously after a very short incubation period (less than 20 hours).

There can, I think, be little doubt that it is indeed these granules derived from spirochætes which enter the erythroblasts of the fowl in which, possibly because of some capsule they secrete, they can be stained by the Romanowsky method. They appear at first as tiny specks and rapidly become the ring or large solid or irregular forms which have been described and which pass on to the "spore" bodies, thus completing a cycle of schizogony or a special cycle, which ends by the discharge of tiny granules (merozoites) into the *liquor sanguinis*. As a result of these later observations I have abandoned the erroneous idea, which I quite failed to prove, that the bodies are directly formed from the spirochætes and that the larger forms are the early forms due to the parasites coiling up and contracting in the red cells. The supposition was perhaps pardonable as spirochætes can and do enter and leave the red cells and the invasion of the red cells by the granules does not appear to take place in any regular manner. There do not seem to be any "generations" of bodies, all completing their cycle of development at or about the same time. The entry and the maturation are apparently irregular as regards time and hence a confusing picture is presented, young forms, ring forms, and spore forms all occasionally being present in one film. A study of some of the case records, however, would seem to give some idea of the usual duration of the cycle. Perhaps renewed study of the infection in the light of these discoveries will yield further information as to this non-sexual cycle. What is its object? This is not altogether easy to answer for one has not yet seen spirochætes develop from the tiny granules set free by the rupture of the schizogony forms in the red cells. True there are indications that this occurs but the mechanism of development as yet remains unknown. Again only a certain proportion of the granules discharged by the spirochætes probably enter red cells. What happens to the rest? From the study of Levaditi sections they appear to be stored up in the internal organs. Is it from these that fresh spirochætes develop and does this in some measure explain the mechanism of relapse not only in this fowl spirochætosis but in human tick fever? Does it also perhaps throw light on the chronicity of such disorders as syphilis and yaws? The granules discharged by spirochætes are resistant, much more so, I think, than the parasites from which they come. One can cure a fowl entirely by "606," presumably by killing all the spirochætes in it outright. If, however, too small a dose be given, granule discharge begins and unless the amount of "606" present is sufficient to kill these infective granules, the cycle of schizogony starts, just as it starts under natural conditions if the leucocytes or the protective fluids or anti-bodies of the bird have not proved powerful enough to destroy all the spirochætes.

A cycle of schizogony

Bearing of the observations on other spirochætal infections

It would seem to be a protective process on the part of the parasites and may also be connected in some manner with the cycle in the tick, but it is little use theorising, and the various questions to be settled will have to obtain their answers by the application of these newer methods and by prolonged and careful observations.

It would seem probable that these infective granules have not previously been noted, partly because they can only be well seen by the dark-field method and partly because when free they do not appear to stain, at least with any certainty, by Romanowsky. This is a very curious thing, but it would seem to be undoubtedly the case.

Importance of the dark-field method

The fact that one is apt to get stain deposit by the Levaditi process and even by its modifications adds to the difficulties of the work, which in any case has shown that it is by the study of the *living* parasite that progress is chiefly made. At the same time the Yamamato modification, slightly altered, has been of signal service.

As this paper has to go to press, it is only possible to indicate along what lines further work is proceeding.

(i) A more extended study of liver, spleen and lung blood by the dark-field method in cases at and following the crisis, to see if the entry of granules into red cells can be confirmed.[1]

(ii) An effort is being made, by using the dark-field method, to discover what becomes of the tiny merozoites set free from the red cells.

(iii) In the same way observations will be conducted on spirochætes ingested by ticks to see if they participate in granule discharge.[2] The development of the Leishman granules in ticks is being studied by the dark-field method. So far it would seem that a meal of blood hastens the change into spirochætal forms.

(iv) The changes, if any, undergone by intra-corpuscular bodies ingested by ticks will be studied in a similar manner.[3]

(v) The infectivity of the peripheral blood, and possibly also that of the blood of the internal organs of birds treated by "606," will be determined.

(vi) It may be worth while to obtain blister serum from infected fowls efficiently treated by "606" and test its value as a curative or protective agent in the same way as has been done in yaws.

(vii) The protective action of "606" will be tested by injecting it simultaneously with the virus.

Summary and Conclusions

The Sudan spirochæte a specific entity

Further work on the spirochætosis of fowls in the Anglo-Egyptian Sudan, while confirming certain earlier observations and conclusions, has led to a modification of some of the previous views. The new features ascertained and conclusions reached may be tabulated as follows:—

1. In all probability the avian spirochætosis of the Sudan is due to a special spirochæte distinct from *Sp. marchouxi* and most, if not all, other species hitherto described with the exception of that recently discovered by Jowett in South Africa. The chief points which have led me, somewhat against my own inclination, for the multiplication of species is to be deprecated, to adopt this view are:—

(*a*) Its peculiar tendency to break up into "infective granules" in the internal organs. These granules, which are discharged by the still living spirochætes from one or other end of its periplastic sheath or cell membrane, would appear to constitute a stage in the non-sexual life-cycle of the parasite, for they seemingly enter the red cells, develop in them, undergo a process akin to schizogony and regain the *liquor sanguinis* as tiny merozoites which vary in number and slightly also in size.

(*b*) The course of the disease in fowls and in chicks. This, it is true, somewhat resembles the spirochætosis of fowls in Senegal, due to *Sp. neveuxi* (Brumpt) and which Bouet found in the French Sudan. If it is the same disease, and there are points of difference, the spirochæte of the Anglo-Egyptian Sudan was first discovered and has priority in nomenclature. Our fowl spirochætosis would appear to differ in some respects, notably as regards the "granule phase" from the Tunisian and Somaliland strains.

(*c*) The peculiar sexual (?) life-cycle, partly worked out in the tick, *Argas persicus* (Fischer) and which seems in most respects comparable to that described by Leishman for *Sp. duttoni* in *Ornithodoros moubata*.

[1] So far, efforts have been unsuccessful in this direction.

[2] They do, both in the case of *Argas persicus* and *Ornithordoros savignyi*, the process being exactly similar to that occurring in chicks.

[3] A discharge of granules from the red cells has been since noted. The granules remained clumped and did not disperse.

(*d*) The absence of any cycle in the tick corresponding to that described by Prowazek for *Sp. gallinarum* (*marchouxi*) in *Argas miniatus*.

(*e*) The fact that lice (*Menopon* sp. ?) have been shown capable of transmitting the disease may be another distinguishing factor.

(*f*) The insusceptibility of pigeons to the disease. The fact that gerbils cannot be inoculated with it may also serve to separate it from other forms of avian spirochætosis.

These then, with other minor points, seem to me to justify one in finally regarding this Sudan spirochæte as a specific entity. I propose to name it *Spirochæta granulosa penetrans* *(nov. sp.), which, if a somewhat lengthy name, would seem to direct attention to its outstanding and peculiar features. I admit that the last word has not been said on what is a difficult subject and await with interest the researches being carried out in South Africa on a spirochæte strain, which would appear to be the same as that with which this paper deals. Meanwhile, recent papers by Bosanquet[1] and Dobell[2] on certain non-pathogenic spirochætes are, to say the least, very suggestive.

Proposed name, *S. granulosa penetrans*

I cannot conclude without expressing my thanks to Professor Ehrlich, who most kindly and generously supplied me with a considerable quantity of his new drug for purposes of experimental research. I am also greatly indebted to Captain R. G. Archibald, R.A.M.C., for his assistance at various stages of the work, and to Captain W. B. Fry, R.A.M.C., for special help in connection with the dark-field work. To Messrs. Buchanan and Marshall, our laboratory assistants, my thanks are also due, and more especially to the former for his admirable drawings illustrating certain of the conditions mentioned; while Mr. F. C. Murray, Chief Sanitary Inspector, Khartoum, has been at pains to procure material for the research in the shape of sick fowls and infected ticks.

Additional Note

Subsequent experiments have proved that if a chick be fed on recently-infected ticks, other means of infection being prevented, it develops spirochætosis. The incubation period is five days. According to the rules of scientific nomenclature the name of the spirochæte would need to be *Sp. granulosa*, and the term *penetrans* reserved as a sub-species title if such were found necessary.

Note on a Method of Obtaining Blood Aseptically for the Culture of Hæmatozoa in the Tropics

by

The Director

Until one hit upon the idea of the simple apparatus herewith described (Fig. 27) one had found it well-nigh impossible to prepare blood-agar tubes for trypanosome culture which did not speedily become contaminated, usually with cocci and moulds. Since it has been in use the difficulty has disappeared, and we have been able in these laboratories to cultivate trypanosomes and Leishmania with comparative ease.

[1] Bosanquet, W. C. (February, 1911), "Brief notes on the Structure and Development of *Spirochæta anodonta*, Keysselitz." *Quarterly Journal Microscopical Science.*

[2] *Loc. cit.*

The apparatus consists of a stout bottle of 500 c.c. capacity, fitted with a rubber cork which is well fastened down and luted with paraffin wax where it is in contact with the mouth of the bottle.

Through this cork, pass two glass tubes, one reaching about half-way down the bottle, the other almost flush with the base of the cork.

The former is connected by means of rubber pressure tubing with a large hollow needle of the kind employed for lumbar puncture. The pressure tubing is controlled by a clamp. The latter is similarly connected with a short length of glass tubing through which the blood is eventually added to the culture tubes. The bottle contains glass beads for defibrinating the blood, and in its neck there is fixed a metal perforated diaphragm to keep back clot.

Method of use. A vacuum is produced in the bottle by means of a Geryk air-pump. Then a couple of test-tubes are fastened over the needle and the glass tubing respectively, as shown in the drawing, and the whole apparatus is sterilised in the autoclave under pressure.

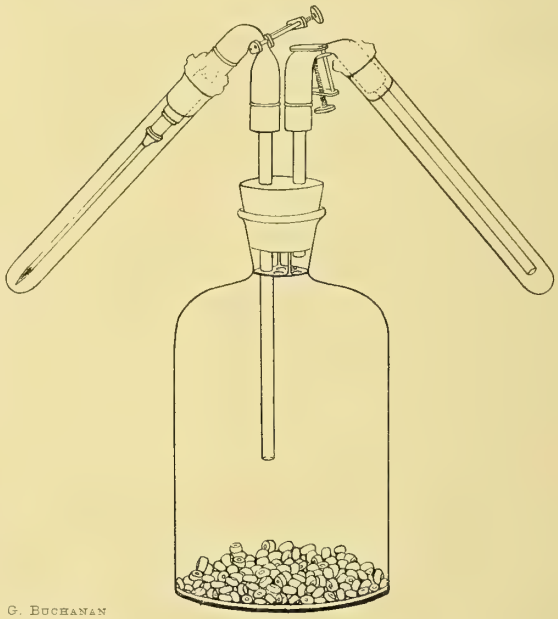
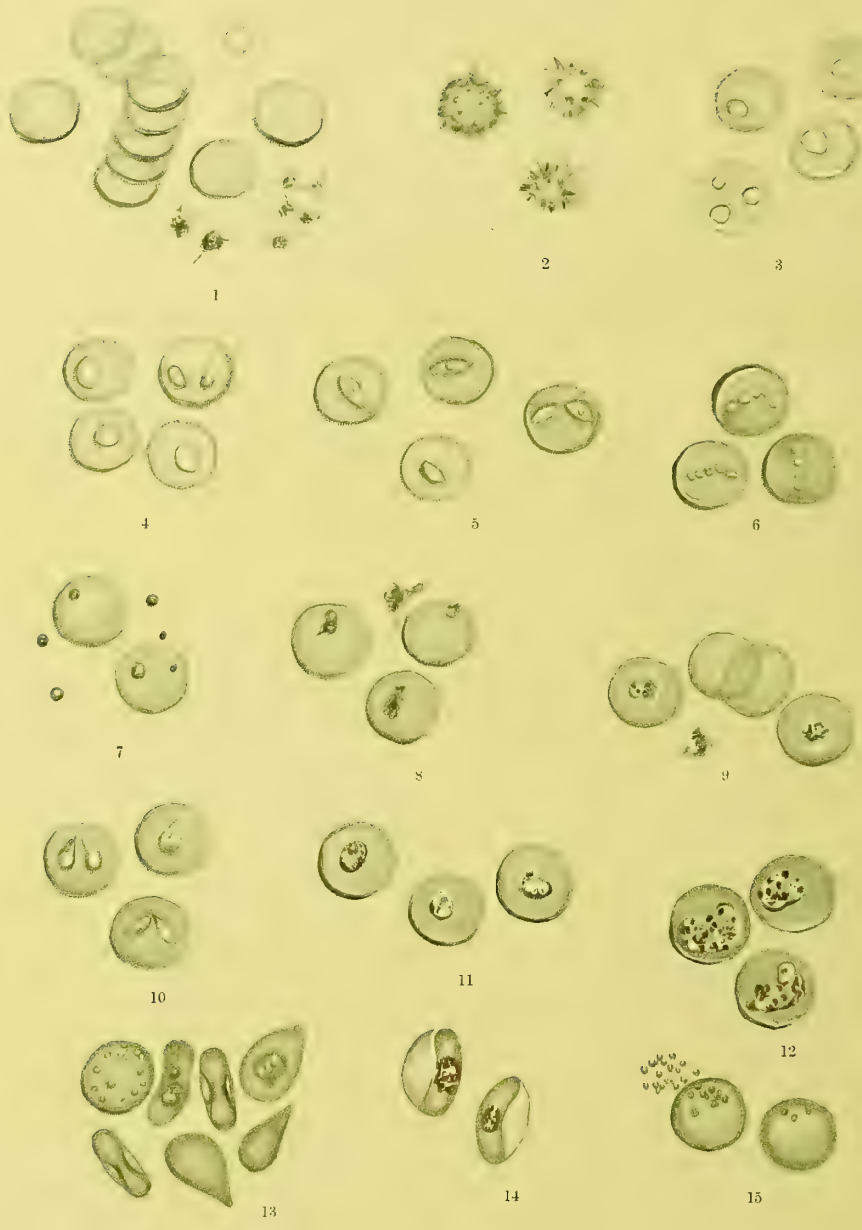


Fig. 27—Apparatus used for obtaining blood, aseptically, for the Culture of Hæmatozoa in the Tropics

A rabbit is now etherised, fastened to a board, its chest shaved, and a point selected in the sixth or seventh left interspace counting upwards from the costal margin. This point is about half-an-inch from the mid-sternal line. The skin over this area is seared with a hot iron. Removing the first test-tube and placing his sterilised thumb against the needle and about half-an-inch from its point, the operator directs the latter downwards and to the left when it will enter the base of the heart just about the root of the great vessels. The thumb-point prevents the needle going too far. The first clamp is then loosened and blood readily enters the bottle which is kept warm by means of a hot cloth wrapped round it. As the blood flows the bottle is shaken to facilitate defibrination. When no more blood can be obtained, the rubber connection is clamped and the needle withdrawn, the rabbit being usually dead by this time. After further shaking, the test-tube is removed from the glass tubing, the bottle is inverted and tubes of melted agar or salt agar have the blood rapidly added to them, the addition being controlled by the clamp or by the thumb and forefinger. In this way, even from our small Sudanese rabbits, some 14 to 18 tubes of Novy-MacNeal or Nicolle's medium can be obtained, and of these one does not expect to have more than two to four go bad through aerial contamination.

I am indebted to Mr. G. Buchanan for the drawing and for some useful suggestions as regards the technique employed.

PLATE V



N. D. Meir

FALLACIES AND PUZZLES IN LIVING BLOOD TOGETHER WITH SOME BLOOD PARASITES BY WAY OF CONTRAST

1. Red blood corpuscles and platelets
2. Red blood corpuscles, crenated
3. Red blood corpuscles showing vacuoles
4. Red blood corpuscles showing spherical and oval gaps or so-called Maraglianos
5. Red blood corpuscles showing eye spots, a special form of fig. 4
6. Red blood corpuscles showing chains, a special form of fig. 4
7. Small globules on red blood corpuscles
8. Granular masses on red blood corpuscles
9. Platelets on red blood corpuscles
10. Piroplasma
11. Young schizont—malaria (pigment)
12. Large schizont—malaria (pigment)
13. Poikilocytes—normoblasts and red blood corpuscles in profile, also one dropsical erythrocyte
14. Gametes—malaria (crescents)
15. Granules from eosinophile leucocytes on red blood corpuscles

× 1000 *diam.*

For convenience one may divide these conditions into two classes according to their origin:—

1. External, Adventitious or Heterogenetic.
2. Internal or Autogenetic.

In the first class we consider cases in which the source of fallacy or the puzzling condition is derived from outside the blood itself as, to take a classical example, the inclusion of insect hairs in a film simulating filariæ. This is the view taken by Low and Stiles of the *Filaria gigas* described by Prout. It is perhaps scarcely necessary to mention another case in which cotton fibres, gaining access to blood films, led to a wholesale diagnosis of filaria cases. This is the type of fallacy which care and cleanliness, the watchwords of a hæmatologist, should avert.

Adventitious fallacies

For several reasons Class I. is more likely to be found in stained blood and it is sometimes far from easy to come to a definite conclusion, especially when one is examining films prepared by another person and perhaps sent from a distance. It may then be impossible to make that examination of the fresh blood film which so often clears up a difficulty. In this connection one would cite the frequent presence of yeast cells in films made in the Tropics. Of this nature are the curious bodies described by Neave, illustrated in the Second Report of these Laboratories and here again reproduced (Plate VI., figs. 1 and 2). The occurrence of chromatin-like granules in such cells is apt to be confusing as is the definite arrangement of a capsule or enclosing membrane and the frequent presence of definite segments. Such bodies are not likely to find their way into fresh blood preparations which have perforce to be quickly made, but they are very likely to be blown by the wind on to films which, though drying, are still wet, and in such a case they may, to all appearance, be on the same level as the corpuscles.

Yeast cells, pollen grains, and debris

Possibly also yeast spores may develop to some extent in blood films and produce confusing pictures.

In the same category come pollen grains and animal and vegetable debris found in dust, but in my experience these are not so common nor so puzzling as the yeast cells. These latter may gain access to blood films in another way, and in this connection I quote a paper which I had occasion to write in connection with the finding by Professor Nuttall, of Cambridge, of a new spirochæte in the African buffalo. Professor Nuttall's original paper, which drew forth my comments, should be consulted, and his drawings—republished here by his kind permission—may with advantage be compared with those I here submit and which represent the similar bodies in the films from the hartebeeste.[1] (*Vide* Figs. 28 and 29.)

I also present Professor Nuttall's interesting reply and, while the question still remains open, would insist on the recognition of this source of error.

Possibility of intestinal parasites in blood films

"I have been much interested in Professor Nuttall's paper in the April number of *Parasitology* (Vol. III., p. 113) and especially in his description of a parasite found by him in blood smears from a buffalo sent to England from British East Africa. He has named this organism *Spirochæta bovis caffris* though he is evidently somewhat doubtful as to its nature. I write to say that early in 1909 I received from Captain Hadow one blood smear from a Jackson hartebeeste which he had shot in the Bahr-El-Ghazal and on the body of which he found *G. morsitans* in the act of feeding. In this smear I found organisms which answered very closely to those described and figured by Professor Nuttall

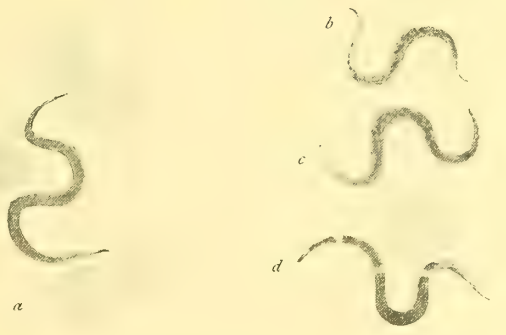
[1] Through the kindness of Professor G. H. F. Nuttall, F.R.S., it has been possible to reproduce his illustration (Fig. 29) of the *Spirochæta bovis caffris* described by him in *Parasitology*, Vol. III, No. 1, April 30, 1910, *pp.* 113 and 114.

(Fig. 28). They were somewhat smaller, having an average length of 16·5μ and a breadth of 0·7μ but presented the same appearance, stained in the same way, and in some instances showed the achromatic transverse bands which he mentions. From its shape I mentally termed one variety the buffalo-horn type and I made drawings of the different forms encountered. On April 18, 1909, I sent the slide to Dr. Wenyon, at the London School of Tropical Medicine, with a note directing his attention to these curious parasites and stating that, to me, they looked more like spirochætes than anything else, but that I was unable to classify them. Unfortunately, though my letter was safely delivered, the box containing the slide was never seen again. Dr. Wenyon, however, wrote and told me that he had come across similar forms in the blood of big game which had been shot, and recorded his opinion that the forms in question were not blood parasites at all but were derived from the intestine and had been carried into the exit wound by the bullet or by discharges finding their way along the bullet track. One recognised the possibility of such an



G. Buchanan × 2000 *diam. (approx.)*

Fig. 28.—Spirochæte-like bodies found in the blood of a Jackson hartebeeste.



× 2000 *diam.*

Fig. 29.—*Spirochæta bovis caffris*, n. sp., Nuttall

a–d Spirochætes showing variation in size but uniformity in shape. Whereas in *(a)* the parasite shows no internal structure, in *(b)* and *(c)* the chromatin occurs as fine dust throughout the faintly violet-blue staining protoplasm. In *(d)* there are achromatic transverse bands visible.

occurrence and the fallacies to which it might give rise, and, on meeting Captain Hadow, I asked him if he remembered where the animal had been wounded. To the best of his recollection, the bullet had passed through the neck, severing the gullet, and it is quite possible that in the last throes stomach contents might have regurgitated through the wound of exit. The presence of very thin thread-like spirochæte forms in the film and of some bodies which suggested yeasts, made me refrain from publishing any account of the case until I had more evidence regarding Dr. Wenyon's hypothesis.

"Quite recently I received from Captain Cummins, S.M.O. Kordofan, a blood smear from a goat which had died from infectious pleuro-pneumonia. He wrote to say that he had found spirochæte-like bodies in the film and solicited my opinion about them. To my surprise, on examining the specimen, I found my hartebeeste parasites or forms very closely resembling them. I wrote to Captain Cummins mentioning the case of the hartebeeste and the view of Dr. Wenyon, and asking him if the blood sent could have been contaminated

from the stomach or intestines. He replied that there was every possibility that this had occurred as, prior to making the film, he had been examining the goat's intestines. Indeed, he thought it very likely that such an accident had taken place and agreed with me in thinking that these parasites were of intestinal origin.

Question of *S. bovis caffris*

"I think with these facts before us we would do well to hesitate to accept *Sp. bovis caffris* as a true parasite of the buffalo. Perhaps Professor Nuttall would kindly state if there were any other peculiar forms, such as yeast cells, in his preparations or anything pointing to infection from internal organs, while it might be well to ascertain if there was any chance of the blood becoming contaminated before or at the time the blood was taken.

"I do not write in any critical spirit but because I know by sad experience that there are many pitfalls in blood work in the open in tropical countries, and it would be well to make certain if Professor Nuttall's interesting parasites are really, as he thinks, *hæmatozoa*, in which case it would appear that the Jackson hartebeeste, of the Southern Sudan, harbours a similar organism. I am inclined, however, to agree with Dr. Wenyon, especially in the light of what was found in the case of the goat, and to consider these curious bodies representatives of the flora of the intestinal tract.

"Perhaps if this letter catches Dr. Wenyon's eye he will give us the benefit of his experience and aid us at arriving at the truth."

Reply.—"Dr. Balfour's note raises the question whether *Spirochæta bovis caffris* is a blood parasite or an organism derived from the alimentary canal owing to the entry of intestinal contents into the blood or blood stream consequent upon the effects of gunshot wounds. I must confess that this source of error did not occur to me in this case.

"On again looking very carefully through the blood-films, I have succeeded in detecting a few small bacilli which indicate that the blood may well have become contaminated either whilst in the circulation or after the films were prepared. Mr. W. F. Cooper obtained the blood as it welled from a stab in the heart of a buffalo (No. 1) which he had shot, the bullet having passed a little in front of the heart and broken the animal's leg. He felt 'sure that there was practically no chance whatever of organisms from the intestines getting into the blood circulation.' On the other hand, it must be noted that the films may have become contaminated after they were taken owing to accelerated manipulations consequent upon another buffalo (No. 2) showing a desire to charge Mr. Cooper whilst he was engaged in preparing the films. I may add that the blood of buffalo No. 2 contained no spirochætes.

"Whether the organism is or is not a hæmatozoon can only be decided by further observations. I am not aware that such forms have been described as occurring in the intestines of animals; they certainly differ very considerably from the typical spirochætes which various observers have found in the intestine."

Parasite-like bodies from the skin and hair of animals may appear in films

Parasite-like bodies may not, however, gain access to blood films only from the alimentary tract. The skin, and more especially the hairy hides in animals, and the feathers in birds often yield their quota, and some very curious looking organisms may thus be added to a blood smear. It is unfortunate that some which I possessed perished in a fire and so cannot be illustrated, but I direct attention to Plate VI., fig. 19, which shows a flagellated form found in a blood film made from a guinea-fowl. I fear I cannot say exactly what it is, but I have a shrewd suspicion it was derived from the feathers. See also the peritrichous ciliate, probably a cyclochæta (Ashworth) from the gills, found by Neave in the blood of a fish (Plate XXI., fig. *a*, Second Report, 1906), and the curious pigmented bodies found by Major Howard Ensor in human blood films taken at Wau. They appear to be artifacts and possibly have an animal origin (Plate VI., fig. 17). In a

PLATE VI



1, 2, 19- R. D. Muir
3 — 18 —G. Buchanan

Fallacies and Puzzles in Stained Blood

1, 2. Unknown and peculiar bodies in the guinea-fowl *(Numida ptilorhyncha)* probably of the nature of yeasts.
3. Plasmosomes of Ferrata—fowl's blood.
4. "Corps en pessaire"—after Brumpt.
5. Filaments in red cells—after Brumpt.
6. Enucleated and distorted red cells in fowl's blood.
7. Greatly altered red cell in fowl's blood.
8. Ring forms (Corps en anneau) in red cells—Webster after Schleip.
9. Curious cells in fowl's blood, probably altered leucocytes.
10. Degenerated leucocyte in fowl's blood.
11. Basophilic red cell with nuclear remnant—after Cabot.
12. *Anaplasma marginale.*
13. Half-moon red cells from case of malarial cachexia.
14. Peculiar bodies in fowl's blood—fragments of thrombocytes.
15. Kurloff's bodies—after Ferrata and Pappenheim.
16. "Jolly" body.
17. Major Ensor's pigmented bodies from Wau, possibly artifacts.
18. Piroplasmosis and Anaplasmosis of the donkey.
19. Pyriform flagellate in blood of guinea-fowl *(Numida ptilorhyncha).*

Leishman Stain

3—19. × 1000 *diam.*
1—2. × 1500 *diam.*

case like this a careful search of the film helps one to a decision, if not to a diagnosis, for it should always be remembered that a parasitic blood infection is usually multiple, that is to say, that it is rare to find only one example of a true blood parasite in any given film. This may occur, especially as regards malarial crescents and filariæ, but if we think we have found a new and peculiar hæmatozoon, and yet can only discover a solitary example of it in, say, two good-sized and well-made blood films, it is well to pass into a mood of combined reflection and doubt. Wherever possible, also, it is advisable, as Daniels says, to examine some part of a slide where the stain has extended beyond the blood film and see if the same appearances are there. Another point worth noting is that in Australia the spermatozoa of birds (honey-eaters) were at first mistaken for spirochætes in the blood, as the long and narrow types show regular spiral curves. The shot wound had apparently injured the spermatic duct and permitted spermatozoa to pass into the blood clot.

Returning to our yeast cells we find they present very varying forms. A glance at Plate XIV., fig. *g*, in our Second Report will show a torula from the stomach of a jerboa which, under certain conditions, might quite possibly deceive the very elect, while reference to *page* 205 in this Report will show what I believe to be another form of yeast cell which was associated with a cutaneous Leishmaniosis, and led more than one person who saw it, astray.

How easy it is to fall into what we may perhaps call the yeast trap is shown by the amount of literature which has accumulated on the parasite of epizootic lymphangitis. This has been definitely shown to be a yeast, and has been cultivated as a fungus growth on artificial media, and yet many able observers have considered it to be a protozoon allied to the Leishmania or Leucocytozoa.

How to distinguish yeast cells from parasites

What then will guide us in distinguishing a stained yeast cell from a blood parasite? Apart from the history of the film and the general shape and appearance of the yeasts, one notices that those portions of their cytoplasm which take on the chromatin hue, stain a very intense ruby-red, and that they are also, as a rule, more compact and "solid" looking than the nucleus or blepharoplast of a hæmatozoon. The appearance of the capsule may help, as witness the well-known double contour of the *Saccharomyces farciminosus*, to adopt Rivolta's name for the parasite of epizootic lymphangitis. In some cases, however, the only thing to be done is to make a careful drawing and record the find with such particulars as may help, especially making mention of any other forms—bacilli, cocci, etc., which may indicate contamination (*vide* Plate VII., fig. 10).

After all, a little experience and careful reasoning will usually prevent mistakes in the case of Class I., but mention must be made of another and most important fallacy. It is, or has been, until recently, a common custom to dry a stained blood film, after washing, by means of blotting paper. What is often done, is to have a bunch of pieces of paper, to lift some of them up exposing the surface of one, place the slide on this and blot the film with those held in the hand. Now, unless care be taken to see that the piece of filter paper, whose under-surface is pressed down upon the film, is of virgin purity, disaster may ensue. I remember telling my laboratory attendant to prepare for transmission to India a number of films of mammalian blood showing hæmogregarine infection. Happily, I bethought me to look them over before despatch. I speedily found they contained oval and nucleated red cells which must have come from a bird or a reptile. Despite denials, I came to the conclusion that either the slides, the pipettes or the spreading needle used had been dirty. It was not until a somewhat similar episode occurred in the course of my own work that I realised what had

"Blotting paper" fallacy

happened, namely, that the same piece of blotting paper used to dry a stained film of fowl's blood had been used to blot the other film and the corpuscles had been transferred from the paper to the slide. I have known of cases in which parasites were transferred in a similar way, so the need of care is obvious. Fragments of stained epithelium and stain deposits often spread beyond the margin of any red cell on which they may lie, and may be troublesome, though focussing helps to decide.

On the whole, however, care and a little experience will soon do away with the fallacies and puzzles belonging to Class I., and with a caution as to avoiding calling dirt or skin pigment, melanin, which latter never occurs free, remembering that cocci and bacteria get into blood and that there are sometimes strange flaws and cracks in glass which may retain a stain, we may pass to the consideration of the second group.

This it is best to consider under two headings:—

1. Those found in fresh blood.
2. Those present in stained preparations.

Autogenetic fallacies

The former, so far as fallacies are concerned, are the more difficult, the latter, I think, contain most of the puzzles.

1. Ordinary crenations of the red cells do not as a rule cause any difficulty (Plate V., fig. 2), but sometimes they present a hyaline appearance and look as if situated in the erythrocytes. Careful focussing soon makes their real nature apparent, for they have no defined outline and change from dark to light or reversely as one alters the focus. In this way they can be readily distinguished from a malaria ring-form, a piroplasm or a spirochætal granule or body, the three things which they are most apt to resemble.

Distorted red cells have been mistaken for malarial crescents, but the absence of pigment should make things clear. I believe a red cell containing a half-grown benign tertian parasite can rupture, or be ruptured, prematurely, and the amœboid processes of the parasite be severed and roll up into little protoplasmic masses in the blood. These are at first puzzling, but the presence of dancing particles of pigment is a sure guide to their origin, and fragments of the broken host-cell can sometimes be seen in their vicinity. Vacuoles are always mentioned as specially difficult, and sometimes they, and also a retraction of the hæmoglobin from the edge of an erythrocyte, cause trouble, more especially in malaria examination.

In distinguishing a vacuole from a young malarial parasite, a piroplasm or a spirochætal inclusion and of the three the last is the most difficult to differentiate, the following points should be considered:—

Vacuoles

(i) Vacuoles usually have well-defined, clear-cut margins, while the periphery of a parasite melts, as it were, into the hæmoglobin of the host-cell (Plate V., fig. 3).

(ii) Although a vacuole may change in shape it never shows true amœboid movement. The slight alterations of shape which may occur in its contour, however, must not be overlooked.

(iii) A vacuole never shows pigment or any appearance suggestive of granules in its interior or projections from the vacuoloid area.

(iv) A vacuole is clear, with no "ground glass" aspect. It is highly refractive and, on focussing up and down, seems to spread and contract in a characteristic fashion.

(v) Vacuoles never stain as a whole, though there may be deposits of colour round their margins.

Splits or cracks in red cells are easily recognised and only require mention, but one of the most difficult of all blood appearances is furnished by areas devoid of hæmoglobin, also called "eye spots," "spherical gaps," "hyaline bodies," "nuclear

fragments," "pseudonucleation," "globular decolorisation," "Maragliano's endoglobular degeneration" or more shortly "Maraglianos" (Plate V., figs. 4, 5 and 6). These, though they are found in normal blood, especially, it seems to me, in that of gerbils and small rats, appear to be more abundant in certain pathological conditions and so are very apt to be mistaken for hæmatozoa. I have little doubt they are responsible for some of the descriptions given by Drs. Foran and Breeze, as they tend to occur in acute infectious conditions, and, as they are found in any part of the red cells, vary in shape and sometimes appear to possess some kind of structure, they may be most confusing. They are not clear like vacuoles, and I have sometimes observed what looked like a tiny waving filament projecting from their centre. This is especially well seen when dark-field illumination is employed. Moreover, though they have no amœboid movement, they seem capable of changing their position in the red cells and this without any pressure upon the cover-slip or disturbance of the blood film. The movement appears to be due to progressing coagulation and consequent constriction of the protoplasm (Webster). They may be multiple and are round, oval and spindle-shaped. The more elongated forms are those which often have a little dark spot in the middle, which may be so distinct as to look like pigment (Plate V., fig. 5). They usually change a little in size on focussing and this helps to distinguish them in some measure. "Maraglianos"

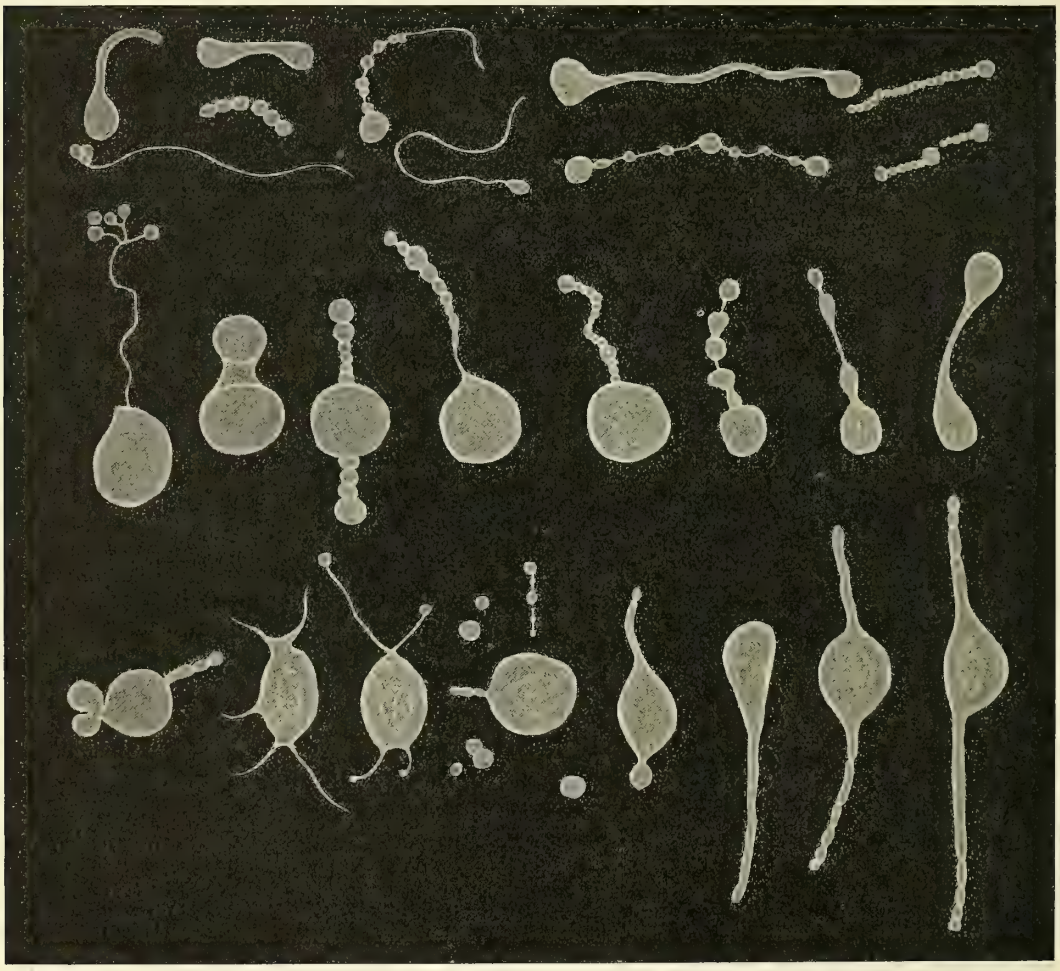
It would seem that the bodies described by Cabot as occurring in pernicious anæmia, leukæmia and lead poisoning, and believed by him to be nuclear remnants, fall into this class, and I expect that further observation on Cropper's bodies met with in cases of fever will show that they are of a similar nature. A glance at Plates i and iii, in Buchanan's book, *The Blood in Health and Disease* (1909), will be found helpful by those to whom abnormal red cells present difficulties. Indeed, this book is very useful to anyone studying blood morphology and more especially cellular degenerations. Half the battle is often to know where to look for information, and British doctors in the Tropics are not likely to turn to Pappenheim or to be regular subscribers to the invaluable but almost too voluminous *Folia Hæmatologica*, edited by that indefatigable German hæmatologist.

Still considering the unstained blood, we must not lose sight of granules from broken-down leucocytes which may overlie red cells and cause trouble (Plate V., fig. 7). They have no amœboid movement, do not present the "ground glass" appearance, and are easily differentiated by focussing, while, of course, they vary greatly in size. It is a good plan, if a doubtful body is seen apparently lying in a red cell, to search the plasma and see if its exact counterpart is not to be found lying free. Granules

While we are on the subject of red cells, it will be well to make mention of the curious degeneration they may undergo, especially when the temperature is raised. The changes which occur have been described and illustrated by Nuttall and Graham-Smith and I have frequently observed them, especially when working with fowl's blood in a thermostat and employing dark-field illumination.

One is inclined to call these alterations in shape "the chain, dumb-bell, droplet, and filament phenomena," a term which, though clumsy, is fairly comprehensive and, I think, descriptive. A glance at Fig. 30 will take the place of a page of print, and it is unnecessary to say more, as, even in the height of a Sudan summer, I have never encountered these changes in blood films examined at room temperature, at least when using transmitted light, so it is very unlikely that they will trouble the ordinary observer, though they are at first very puzzling to anyone working with heated blood. As Nuttall and Graham-Smith point out, however, these alterations in red cells may be produced "Chain, dumb-bell, droplet, and filament phenomena"

if one employs a platinum loop to transfer a drop of blood to a slide and does not take care to see that it has cooled sufficiently after sterilisation in the flame. The glow may have faded from it but it still may be too hot. The same thing may occur if a finely drawn glass pipette is used immediately after it has been made, so it is well to be careful in these directions.



G BUCHANAN

FIG. 30.—ALTERED RED CELLS

The chain, dumb-bell, droplet, and filament phenomena in fowl's blood and human blood as seen by the dark-field method
In part after Nuttall and Graham-Smith
Not drawn to scale

In the fresh blood of man the platelet is not very likely to be a source of fallacy, but it is said that a platelet lying on a red corpuscle may simulate a malarial plasmodium and is to be distinguished by the fact that it is less opaque, has a granular look and is less refractive. Moreover it often presses the hæmoglobin of the red cell away from under it and so looks as though surrounded by a pale area (Plate V., fig. 9). It may here be pointed out that in the case of any doubtful body lying on, or apparently in, a red cell, help will be obtained by altering the illumination of the field. A dull light is responsible for many errors and brighter illumination may even change the colour of a dark object from black to a bright crimson. By touching the edge of a cover-glass with a needle

and seeing if the body moves with, or independently of, the red cell, error may sometimes be avoided.

In the blood of cattle especially, platelets may assume strange shapes suggestive of flagellates, but the fact that these thrombocytes always tend to unite in clusters helps one to distinguish them. Both in human and animal blood there is likely to be more trouble with platelets in stained preparations (*vide infra* and Plate VII., fig. 2).

At the same time a mass of platelets in human blood, surrounding a little dirt which simulates pigment, may look very like a sporulating malarial parasite, but blood platelets have ragged edges and one can nearly always have recourse to staining in a case of this kind, while one should never dissociate the blood examination from the clinical conditions. It is perhaps worth noting here that the latest work on the histogenesis of blood platelets goes to show they are detached portions of the cytoplasm of megakaryocytes, *i.e.* the giant cells of blood-forming organs (Wright). Swingle has shown that blood platelets in blood ingested by an insect host may very closely resemble herpetomonadine flagellates and, from what I have seen, I believe errors can arise in fresh blood if this fallacy is not borne in mind. It is, however, rather beyond the scope of this paper to consider difficulties in connection with ingested blood. No one should take up this study until he is in a position to grapple with the numerous problems which assuredly await him. The same is true of the examination of blood from culture media.

For those who seek to diagnose malaria from the presence of free spores in fresh blood, a proceeding which Deaderick emphatically condemns, the little dancing particles of blood-dust or hæmoconia may serve as a stumbling block. Personally, I do not think blood-dust ever simulates parasites though it may be confused with granular debris from leucocytes, Hæmoconia a matter of no moment, or with micrococci or fat droplets. The latter would appear not to be an error, for, though Buchanan says they consist of fibrin, recent work with the ultra-microscope by Neumann and others goes to show that they are really fat particles possessing Brownian movement. They are more numerous after a meal and disappear if the person or animal is starved. If necessary, in the case of animals, this would furnish a means of distinction. They may be absent in persons suffering from disorders of the alimentary tract. Some of the appearances last described, and more especially blood-dust and droplet degeneration of red cells, are much better seen with dark-field illumination, and as the day will come when this method will be employed as a routine procedure, and also, in all probability, as a method of differential diagnosis, some words on the appearance of microscopic fields observed under this condition may not be out of place. The first thing that strikes one is the enormous number of minute dancing particles. These vary in size from about 2μ in diameter to the very tiniest specks which, being brilliantly illuminated, look like infinitely minute sparkles. Crawley states that even with a magnification of 2,160 diameters some look only like tiny points of light. The larger particles vary in shape being round, oval, rod, or dumb-bell shaped, and, while the smaller are solid, the larger may appear vesicular. In these, which are highly refractile, one can sometimes see a central spot of a ruby colour. This is mentioned by Porter. Crawley describes particles exactly like blood-dust, which certain focussing tests and observations on their limited movements apparently proved to be intra-corpuscular. I have never noticed these. He thinks some of the blood-dust must therefore consist of broken-up erythrocytes or fragments derived from them, but, personally, I do not see why fat particles by a process of osmosis might not find their way into red cells. Indeed, in ordinary fresh blood films, viewed by transmitted light, I have seen spherical bodies in red cells which certainly suggested oil globules. It is worth noting that according to Love, hæmoconia are specially numerous in the blood

of typhus fever patients. One has mentioned the chain, dumb-bell, droplet and filament phenomena exhibited by red cells brought under the influence of heat (Fig. 30). Crawley has recently described similar changes seen in blood with dark-field illumination and has termed them beaded threads. I have also observed these and I was at first inclined to think that the proximity of a hot lamp, the source of light, had something to do with their presence, but recent work with a better apparatus has negatived this view, and it is evident that they can be seen by the dark-field method when there is no possibility of the blood temperature having been raised, at least to any appreciable extent. Crawley also noted free flagella in the blood. These are probably of the same nature as the beaded threads, and are, I expect, what I have termed filaments, but one must not forget that under artificial conditions lymphocytes (Ross and Macalister) and myelocytes (Buchanan) may flagellate. Such a phenomenon may be induced by certain excitants, atropine and methylene blue, as well as by a cancerous plasma. This being so, it is possible that certain pathological conditions of the blood may bring about the flagellation in white cells and that free flagella may account for some errors of diagnosis. Indeed, Crawley, I find, mentions their occurrence. Bodies showing pseudopodia have also been seen in normal blood under dark-field illumination, and thereby a fallacy was cleared up, for at one time they were supposed, by Koch and Kleine, to be associated with piroplasmosis in dogs and cattle. They are only greatly altered blood cells, and Crawley believes them to be more probably derived from erythrocytes than from leucocytes. Crawley has also some interesting notes as regards the behaviour of both red cells and white cells under this form of examination, and I see he notes the stimulating effect of the heat of the dark-field method upon the leucocytes. The vesicles which he describes as occurring in red cells are, I think, analogous to the bodies I found in the fresh blood of fowls and which I fear I mistook for a special form of intra-corpuscular spirochætal inclusion (*Journal of Tropical Medicine and Hygiene*, October 1, 1909). I have certainly seen such bodies grow smaller and become the nebulous clouds of which he speaks. He can give no explanation of this phenomenon, and neither can I, but I was able to recognise I had made an error by finding similar bodies in the blood of gerbils, which, though inoculated with spirochætal blood or blood supposed to contain spirochætes, never showed any sign of illness. The trouble is that the true intra-corpuscular forms look exactly like the pseudo forms. I believe the fact that the latter are motile, a point mentioned by Crawley, may serve to distinguish them. Reference to this matter will also be found in the paper entitled "Spirochætosis of Sudanese Fowls," *page* 84. Blood plates are better studied by transmitted light, but it is said that the dark-field method has shown that mononuclear leucocytes break up into bodies like hæmatoblasts, an observation which, if confirmed, while not of much importance in the examination of fresh blood specimens, may well explain some puzzling features met with in stained films of animals' and birds' blood.

Free flagella

A suggestive paper is that on leucocyte degenerations in the cerebro-spinal fluid by Eve. The illustrations he gives show that if similar changes can occur in the blood, and under certain conditions nothing is more likely, then there is every probability of the resulting motile, globular, hyaline and flagellated bodies which are produced, being mistaken, as they were at first by him, for extraneous protozoal contaminations.

Fallacies in stained blood

2. We pass now to the examination of fallacies and puzzles in the stained blood, and as we are here only considering those of an autogenetic nature, it will be as well to take up in turn each of the main groups of blood elements and see how it may deceive or trouble the observer. Pride of place may be given to the little blood platelets, for I believe they are a more common source of error than anything else in the blood.

A blood plate resting on a red cell may, as in fresh blood, cause the inexpert to diagnose malaria, but the fact that it has no blue-staining protoplasm, that its chromatin is deep red, irregular and granular, that its outline as a rule is somewhat indefinite, and that generally it has pushed the hæmoglobin away from below it and consequently produced a ring-shaped, non-staining area around it should prevent the error (Plate VII., fig. 2). A mass of platelets has before now been mistaken for a crescent or a clump of spores, but the staining reactions and the lack of pigment should leave no room for doubt. I have seen platelets which required a little careful observation to be sure they were not Leishman-Donovan bodies, for they sometimes show dark chromatin rods very like blepharoplasts, but one is guided correctly by much the same considerations which weigh with one in distinguishing them from malarial plasmodia. Their reticular structure is, I think, on the whole, the surest guide to their identity. Osmic acid fixation probably preserves their form better than any other method.

Platelets

In animal blood the platelets may assume confusing forms as may be seen by reference to Plate XV. of our Second Report where flagellated and blue-staining forms are seen. A little experience soon sets one right. The same thing applies in a lesser degree to the platelets in equines. It is not, I think, necessary here to enter into the question of true and false platelets and the so-called Arnold's bodies, but as a red cell is often seen apparently extruding platelets—an appearance which may confuse a beginner—it is worth noting that Hugh Ross states that this phenomenon is never apparent when blood is examined by *in vitro* methods. He has also drawn attention to the presence of diffusion vacuoles in platelets which appear as red spots when stained. In fowls, the so-called thrombocytes take the place of platelets, and as they are quite large cells and may stain quite deeply by the Romanowsky method, I have known them mistaken for hæmogregarines (*Third Report*, Plate V., fig. 2). I cannot do better than quote the description given by Burnett of these *spindelzellen*, as von Recklinghausen named them, especially as I find very few people, even those accustomed to blood work, seem to recognise them as a distinct entity. They are most likely to be mistaken for distorted lymphocytes.

Spindelzellen

Burnett describes them as "elliptical, oblong or spindle-shaped cells with an elliptical to broadly oval nucleus. In size the cell has nearly the length and about one-half the width of the average red cell. The nucleus occupies about one-half the length and nearly the entire width of the thrombocyte and is usually situated in the central part of the cell. The cell body is pale and often contains one or more clear vacuoles and occasionally one or more compact, rounded, deeply staining (deep purple with Wright's stain) bodies about the size of, or somewhat larger than, a mast cell granule. These bodies are probably a result of degeneration. The thrombocytes show a marked tendency to collect in clumps. In fresh blood and in the less thinly spread parts of films, they collect in masses in which it is difficult to distinguish the outline of individual cells. This indistinctness of cell outline and structure shows another property of these cells, that is their vulnerability. They change quickly when taken from the blood vessels, passing through a characteristic series of changes. Both cell body and nucleus become less distinct, the cell body losing its structure first. Finally, both become structureless, appearing in stained preparations merely as a diffusely stained mass, the nucleus being distinguishable by having a slightly deeper stain."

Alterations in red cells

Turning now to the red cells, alterations in shape and size need not long detain us, for all the ordinary changes are fully dealt with in the standard works on the blood and are, or should be, known to every medical student. When, however, an observer for the first time encounters in the blood of a fowl forms like Plate VI., figs. 6 and 7, he is apt to be puzzled although they are merely distorted and enucleated erythrocytes which

have probably assumed these shapes as the result of the spreading of the film. Plate VI., fig. 7*a*, shows another curious appearance due to this cause and which the inexperienced might mistake for a parasite. So-called hæmoglobin drops are also met with in birds' blood. They look like human red cells. Again the *corps en pessaire* and *corps en anneau* of the French authors, Plate VI., figs. 4 and 8, are not always correctly diagnosed by the student of the blood of malarial cachectics though their colour should prove their source. Fragile red cells assuming half-moon and other peculiar shapes are apt to be confusing. I have seen them in chronic malaria (Plate VI., fig. 13).

Corps en pessaire

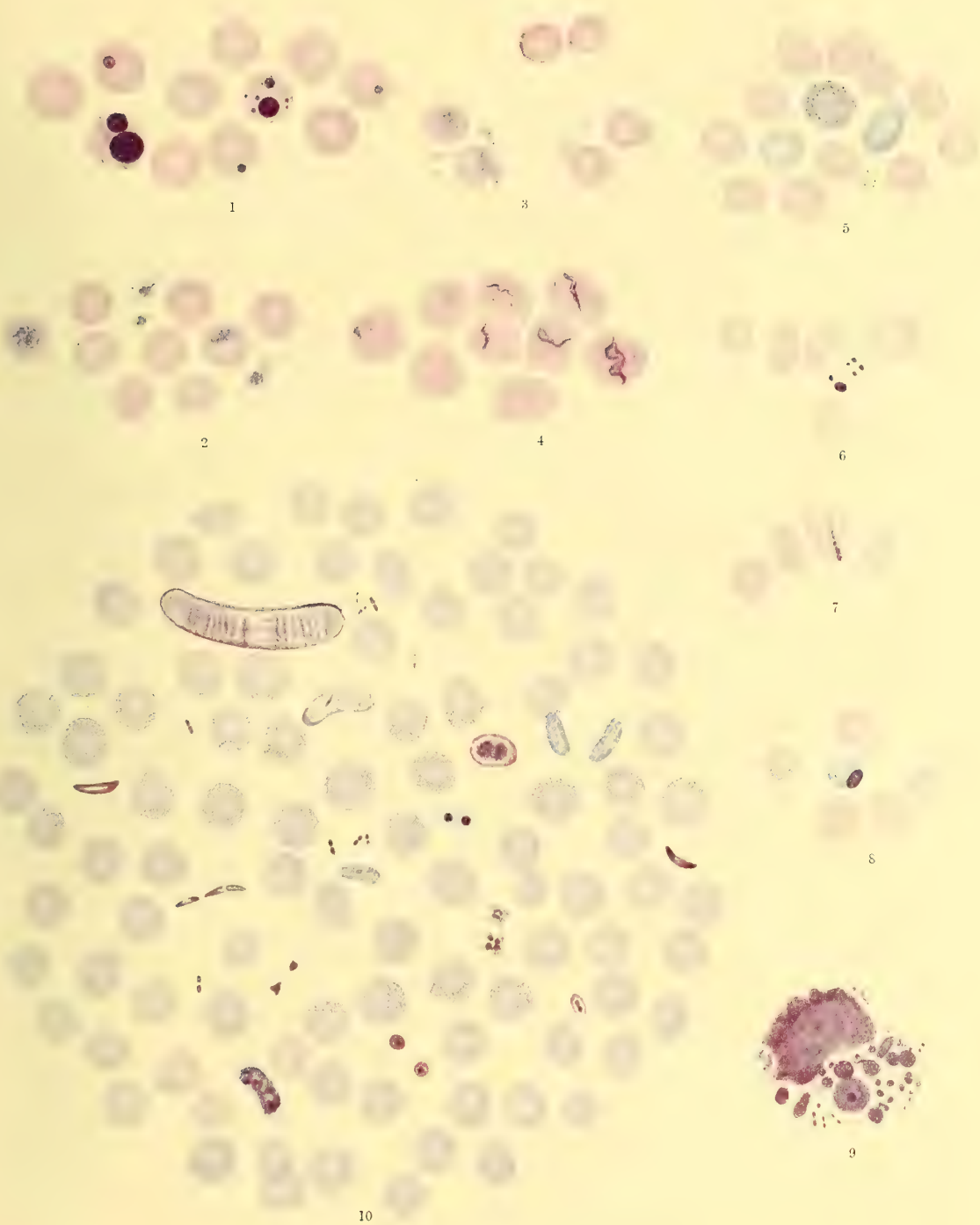
When one encounters endoglobular and anæmic degeneration of red cells with a deposit of blue stain at their centres one may go wrong and think of malaria or a new hæmatozoon, but the absence of chromatin and the general distribution of the stain are distinctive. There is more reason for granular or bacillary-like basophilia (Plate VII., fig. 5), especially when associated with polychromasia, leading one astray. Basophilia is very common in the blood of all kinds of rats, and, indeed, some persons think that certain so-called basophilias are not histological changes at all but are due to parasitic invasion. For instance, Graham-Smith, in 1905, described bacillary-like bodies with protozoal staining reactions in the red cells of the mole. These are not at all unlike the rod forms I found in the jerboa and which Laveran assured me merely represented basophilia. The latter, however, do not usually stain blue with Romanowsky, and in cases of this kind the tinctorial reactions are of the greatest service in arriving at a diagnosis. They may, however, do so. There are also different kinds of granules. Nægeli's azurephil granules, which are probably due to degenerative changes in old erythrocytes, are quite distinct from what Ferrata calls the true blue basophilic punctations which have a regenerative significance and consist of what Pappenheim calls parachromatin, a substance allied to the chromatin of which the Jolly bodies (*vide page* 121) are composed. Mention may also be made of Cabot's ring bodies which are rare and occur in acute, large lymphocyte leukæmia and pernicious anæmia. They stain red both with Romanowsky and with methyl-green-pyronin. Sometimes true basophile granules may be very large like those met with in embryonal blood. Recent work on staining the living blood has led to the discovery of the granular-filamentous substance of Cesaris-Demel and his metachromatic-granular material, but in ordinary blood work consideration of these can, I think, be discarded so far as our present point of view is concerned. It is worth noting that the Maurer and Schüffner dot phenomena of malaria are placed in a special class by Pappenheim.

Curious appearance in gerbil's blood

Plate VII., fig. 7, shows an appearance noted in a polychromatophilic red cell from a gerbil which had been inoculated with finger blood from a relapse case of human spirochætosis but which never showed infection. It will be seen there was a curious chromatin streak which ends in a kind of short flagellum, running down the centre of the corpuscle. What is this? I confess I do not know, but think it must be a stain deposit in a corpuscular split or crack. It was the only thing of the kind in the film. It is probably allied to the ring and figure-of-eight bodies (*corps en anneau*) mentioned by Schleif, Webster, Brumpt and others, or to the intra-corpuscular polychromatophilic filaments known to occur in red cells (Plate VI., fig. 5).

Very frequently in the blood of rodents, especially rats and guinea-pigs, stained by the Leishman or Giemsa methods, one encounters little spherical chromatin dots in the red cells, recalling in all but position the marginal dots (*Anaplasma marginale*), Plate VI., fig. 12, of Theiler, now said to be the parasite of gall-sickness in cattle in South Africa. To the best of my knowledge, one may search in vain through English books for any

PLATE VII



R. D. Muir

FALLACIES AND PUZZLES IN STAINED BLOOD

1. Blood of donkey showing anaplasmosis of red cells and a curious spherical body of unknown nature, possibly associated with an accompanying piroplasmosis.
2. Blood platelet lying on red blood corpuscles.
3. Stain precipitate on and around red blood corpuscles.
4. "Chinks" or cracks in red blood corpuscles.
5. Basophilic rods, free and endoglobular. Polychromasia of red cells.
6. Blood film from gerbil which had been inoculated with blood from goat unsuccessfully inoculated with camel trypanosome blood. Curious bodies with spherical granules.
7. Blood film from gerbil showing chromatin streak in red cell.
8. Blood film from gerbil which had been inoculated with human spirochætal blood. Unknown ring body.
9. Macrophage, or leucocyte undergoing chromolysis.
10. Blood film from red or Uganda cob showing contamination from yeast cells and other curious bodies simulating parasites.

Leishman Stain × 1000 *diam.*

account of these, and I am quite sure they have perplexed many a student in the Tropics. These little red bodies were first described, so far as I know, by Jolly, and bear his name alone or are called Howell-Jolly bodies, Plate VI., fig. 16. Ferrata has made them the object of study. They are most commonly single, but two may occur, one often being larger than the other, and they may be combined with punctate basophilia. It is important to note that they have been seen in human blood in cases of leukanæmia. A study of the normal bone-marrow apparently showed them to be chromatin residues from nucleated erythrocytes, but Jolly has recently put forward the view that they are probably due to hydration of the stroma or corpuscular membrane, and believes that polychromatophilia, granular red cells (seen in unfixed films) and basophilic granulations are only different degrees of the same change. One need not go further into this question here beyond pointing out that a study of embryonal blood clears up many obscure points in blood cell morphology. These nuclear remains, if such they be, stain red with Leishman and Giemsa, and green with methyl-green and pyronin combined. A very puzzling conjunction, and one frequently overlooked, is a nuclear fragment situated in a basophilic red cell which stains blue like a leucocyte fragment (Plate VI., fig. 11).

"Howell-Jolly" bodies

Until quite recently the marginal dots of Theiler, found in cases of piroplasmosis in cattle, were doubtless thought by most to be of this nature, though Theiler himself could offer no explanation regarding them. Now, however, he and Spreull believe them to be of a parasitic nature, and he has named this new protozoon *Anaplasma marginale*, and apparently definitely proved it to be the cause of gall-sickness (Plate VI., fig. 12). According to Spreull they may occur free in the plasma. Whether or not this work is confirmed, it behoves all workers in the Tropics not lightly to dismiss chromatin-staining bodies in red cells as nuclear remnants, but to find out if they may have any clinical and pathological significance, to look for them in the fresh blood, to test the effects of different staining methods upon them and to carry out suitable inoculation experiments. More especially is this true if they appear to have a selective affinity for any special portion, *i.e.* the margin, of the host cell. (*See also page* 345 of this Report.)

Megaloblasts showing polychromatophilia, and especially if their nuclei are undergoing degeneration, may be a source of trouble, but the presence of ordinary megaloblasts in the film should clear things up just as the free nuclei of normoblasts are explained by the presence of nucleated reds. In the red cells of birds and reptiles one may encounter the extra-nuclear centrosomes of Ross, Moore and Walker. They have also described them in human corpuscles. They stain bright red with polychrome methylene blue and occur as single granules, or as little groups of granules often connected by filaments. The result is a kind of star rosette. They occur along with the chromo-linin granulations of these authors and are shown by their methods of vital staining. Ferrata and Boselli have recently described the chromo-linin granulations under the name of the granular thread-forming (fadenförmige) substance.

Centrosomes in bird's erythrocytes

It is now time to turn to the white cells and see what they can yield in the way of fallacies and puzzles in stained blood preparations. I do not here intend to say anything about the leucocytes of pathological significance, such as myelocytes, which may be present in the peripheral blood, or to describe different kinds of basophiles, mast cells, etc. It seems to me that these are rather outside the scope of this kind of paper, and in any case they can usually be recognised without much difficulty. One wishes rather to deal with fragments from white cells, inclusions in them, curious nuclear changes and conditions which may suggest blood parasites either in leucocytes or free in the plasma and yet derived from them.

For instance, the spherical, homogeneous, blue-staining bodies, so often met with in spleen smears in kala-azar, may be found in the peripheral blood, and to those not accustomed to them prove a difficulty. They are, however, quite distinctive, especially if one remembers that in all probability they are fragments of host cells (macrophages) which, having been broken off, assume the spherical shape in the circulating blood. This, however, is by no means proved, and what is one to make of the body shown in Plate VII., fig. 1, or that portrayed under fig. 6?

Unrecognised bodies from the blood of a donkey and a gerbil

The first is from the blood of a donkey which was possibly a recovered case of piroplasmosis, the latter was from the blood of a gerbil which had been inoculated, with negative results, from a goat, which in its turn had been inoculated with the camel trypanosome of the Sudan.

I confess I do not know what these bodies are, and I would be glad of suggestions. The first is, possibly, not unlike the bodies into which mononuclear leucocytes may break up *(vide page* 118*)*, and it is worth remembering that in severe and advanced cases of piroplasmosis, whether bovine, equine or canine, the blood may show marked degenerative changes and present curious pictures of cellular vagaries. From the same donkey's blood comes Plate VII., fig. 9, and it is almost certainly merely a degenerated leucocyte where the nucleus has undergone chromolysis. In the same category come, no doubt, the strange cells, Plate VI., figs. 9 and 10, from the blood of a fowl which was dying in what I have called the spirochætal "after phase," or better, "granule phase." There were myelocytes present in its blood and every evidence of great blood destruction.

The work of Ross on achromasia of the leucocytes and on the changes which occur when they die, helps us to understand some of these appearances. After their death polymorphonuclears and eosinophiles often appear mononucleated. This is possibly due to liquefaction of the nucleus, its lobes running together. Liquefaction of the cytoplasm is said to begin at the periphery of the cell, and progress towards the nucleus, while in achromasia the granules of leucocytes turn red. It is well to keep in mind the fact that in blood films we may possibly encounter white cells which had been circulating as dead matter, and, therefore, exhibit changes which are never seen in living cells.

Death and degeneration of leucocytes

Apart from actual death, degeneration and vacuolisation may make leucocytes almost unrecognisable, especially if there is much karyolysis of their nuclei. The clinical condition, the history of the case, other blood findings and the evidence of grave anæmia will usually lead one to a diagnosis of such abnormal cells. Anyhow, they cannot well be mistaken for anything else, and their outlines are too irregular and broken for them to suggest protozoa. Plate VII., fig. 8, however, represents a parasite-like body, about which I cannot give an opinion, though I expect it is derived from a white cell. It was found in the blood of a gerbil which had been inoculated, without result, with human spirochætal blood. The chromatin is well marked in it, and, as will be seen, it is ring-like.

Although not like these, this leads one to speak of oval or round rings which may occur in the cytoplasm of lymphocytes. Certain forms, which Ross suggests may be centrosomes, occur when blood is kept on a jelly and stained with an alkaline stain. They are situated above or below the nucleus, not in it. Is it possible that such rings may show, under certain conditions, in the peripheral blood? I have seen in the extra-nuclear portion of a camel's mononuclears, tiny chromatin-like and very perfect rings, the significance of which is quite unknown to me. They were not numerous, and

I do not think they had anything to do with the trypanosomiasis from which the animal was suffering.

If "ruby" granules are found lying free in a blood film stained by Leishman's method one may be pretty certain they are derived from a lymphocyte, at least if the blood be human, but I do not think anyone would make a mistake about them, and they are not common, for the lymphocyte is not a very friable cell. They stain quite differently from free eosinophile granules which only a tyro would fail to recognise at a glance. It must not be forgotten that recent blood investigations have shown the frequent presence of special granules or bodies in the extra-nuclear cytoplasm of certain of the mononuclear elements. The most important of these are those known as Ferrata's plasmosomes, Plate VI., fig. 3, and Kurloff's bodies, Plate VI., fig. 15. The former are small, are found in all types of lymphoid mononuclears, possess what is called a metachromatic staining reaction, and by Ferrata are thought to be evidence of a regressive metamorphosis. The latter, which are larger, and which have been the subject of a special paper by Schilling, have been supposed to be secretion vacuoles or—and from the point of view of the present paper this is important—parasites. Indeed, I have seen it suggested that the intra-corpuscular stage of fowl spirochætes, which I described in red cells, was in reality of the nature of plasmosomes or Kurloff's bodies, hence the importance of bearing them in mind.

Bodies in the extra-nuclear protoplasm of certain mononuclears

Schilling's work goes to show that they are merely phagocyted structures (the action having taken place during blood formation), which undergo only regressive change after absorption by the mononuclear cell. His observations were made on the blood-forming organs and tissues of the guinea-pig. An illustration of these bodies is shown, Plate VI., fig. 15, which I trust may be helpful. I have encountered them in the mononuclears of fowls and rats, and for a long time did not know what they were.

One has mentioned fowls' blood, and some reference is necessary to the interesting work of Kasarinoff on the blood of pigeons. (*Folia Hæmatologica*, Bd. X. H. 2. Part 1. Archiv. November, 1910). His fine plates of normal and abnormal cells, and curious blood findings are well worthy of close attention. Of special interest are the polar bodies he describes in thrombocytes after treatment of the blood with saponin. They appear as small, single dots, or as spherical solid bodies, or as rings with solid inclusions, or as signet shapes, or as little spore-like clumps. Indeed, save for their colour reaction ("azurephil") and the fact that they occur in thrombocytes they closely resemble some of the bodies in fowl's red cells which I believe to be spirochætal inclusions. As I state elsewhere (*page* 85) it may be that one has confused two conditions, and that some of the red cell forms are of the nature of these peculiar polar bodies. So far as I know the nature of these has not been settled.

Kasarinoff also draws attention to the spherical plasmocytes of birds' blood which are like human blood plates and are portions torn off the plasma of thrombocytes (plasmorrhexis). Their likeness to blood plates is intensified by the fact that they may contain remnants of the polar bodies at their centres. I have seen these in films showing fowl spirochætosis and they are shown in Plate VI., fig. 14.

Spherical plasmocytes in bird's blood

A paper in English which gives some account of the red and white blood cells of certain animals is that by Goodall (*Journal of Pathology and Bacteriology*, Vol. XIV., p. 195, 1910) which may be found useful by laboratory workers in the Tropics, though it must be remembered that the state of the blood in hot countries may differ very considerably from that in temperate climates. Unfortunately, we have no tropical standards to guide us, at least as regards the lower animals.

There are many other points with which one might deal, including conditions met with in smears from such organs as the spleen and liver, but I feel that a paper of this kind should not be too cumbersome and, after all, the ordinary clinical and laboratory worker does not find it necessary as a rule to plunge very deeply into the abstruse aspects of hæmatology. In any case he will find the *Folia Hæmatologica* a mine of wealth, and realise how very far we are behind the Germans in a full knowledge and right understanding of well-nigh everything connected with the blood. Our text-books, though practical, avoid altogether those doubtful subjects which are often those on which light is required, and I trust that some one with time, leisure, and the necessary knowledge, will produce an English work which may aid those of us who have little time to read lengthy German papers, who have to work both at human and animal blood, and who are constantly meeting with appearances which we either cannot explain or class vaguely as degenerations.

Uses of the *Folia Hæmatologica*

It may, however, be as well to make mention of a few recent and little known discoveries of true hæmatozoa, either in human or animal blood so that the worker in the Tropics may be on the look-out for these or similar parasites.

Taking the human blood first, there is the ectoglobular, vermiform parasite found by the brothers Sergent in Algeria and now named by Brumpt *Sergentella hominis*. It is something like the leucocytozoon of a bird in shape, being pointed at the ends and containing a large oblong nucleus at its centre. These parasites exhibited periodicity, disappearing from the blood in the evening, and may have been the cause of the nausea and night sweats from which their human host suffered. In some illustrations it looks not unlike Nuttall's buffalo spirochæte and my hartebeeste forms. Somewhat crescentic and vacuolated bodies found by Castellani and Willey in human febrile cases and by Castellani and Sturgess in *Bos indicus* also merit consideration.

Then there is the unknown protozoon found by Hoefer in a case of anæmia at Leipzig, in a woman who had never been away from the neighbourhood of that city. It stains like a malarial parasite, shows chromatin, occurs in the red cells as rings or as curved pyriform bodies, and also apparently as a large blue staining mass, dotted with fine chromatin granules and rather like a benign tertian parasite, but devoid of pigment. In the plasma, double, possibly dividing, free forms occur which may show two nuclei each, and are either pear or somewhat spindle-shaped. There is also another free form something like a stout vermicule.

It would be well to bear in mind the possible occurrence of hæmogregarines in human blood of the type found in certain mammals.

"Howell and Horrocks" bodies

One does not know what the X bodies of Howell and Horrocks are, but, as they seem to be associated with febrile conditions, they may be of a parasitic nature, though, if so, they are something entirely novel. I have seen them once in the blood of a sick dog, and the following is the description given of them when stained with Leishman's stain:—

The bodies, when stained, were characterised by a faint capsule with a circular centre, staining deep blue; they varied in size, some being as large as a red corpuscle, others only about one-eighth the size of a red corpuscle. In addition to these forms, which were the most common, the following were also seen:—

(*a*) A small, blue circular centre surrounded by four or more faint capsules concentrically arranged; (*b*) two circular bodies, each having a dark blue central point surrounded by a light blue ring enveloped in one capsule which appeared indented as if two capsules were in process of formation; (*c*) similar to (*b*), but the part surrounding the deep blue centre stained a deeper blue, and two indented capsules were

seen; (*d*) a dark blue central part, shaped like a crescent, containing a small circular body, with a deep blue central point within the arms of the crescent. None of the bodies on the slide showed any signs of chromatin. Since the above was written I have found them in human blood in a case of urticaria without any fever (*vide page* 362). Major Howard Ensor has also come across them in a case of fever and in a splenic puncture film from a case of supposed kala-azar.

Unidentified parasites in frogs' blood

As regards parasites in animal blood, certain unidentified forms in frogs' blood claim attention. Thus there is one form found in this amphibian's red cells and described by Dutton, Tod and Tobey. The appearance is that of more or less spherical chromatin staining bodies, and recently Carini, who has been working with frogs' blood in Brazil, has suggested that it represents an endoglobular stage in the life history of a trypanosome, for he has found and figured such a condition. Certainly at an early stage the small inclusions he illustrates resemble the bodies described by the Liverpool observers. I think, however, that the so-called cytamœbæ, at least in certain of their forms, which these latter also discovered in frogs' blood in West Africa, are even more like Carini's early trypanosome stages, and also closely resemble, as I have mentioned elsewhere, some of the "after phase" bodies in the blood of spirochætal infected fowls. Other cytamœbæ in the frog, however, approximate to the "bacilloid" and bacillary bodies which Carini also found in *Leptodactylus ocellatus*. The first are little oval, blue-staining masses with a single central chromatin dot, spherical or star-shaped, or two granules, one at either end, or a splash of chromatin along their sides. They may be single and multiple, the latter being apparently division forms.

The bacillary forms are perhaps of greater interest. Kruse was the first to find them in frogs caught in the neighbourhood of Paris, and their frequent presence in the red cells of European frogs has been signalised by Labbé, Laveran, Ziemann and others. They look for the most part like little bunches of bacilli irregularly arranged at one end of the erythrocyte and having no connection with the nucleus. They may have a beaded look or may appear almost like a tangled skein in a vacuoloid space. They may also look like rods sticking out of a central dark-staining mass. Those found by Carini are shorter and thinner than those called *Bacillus krusei* and are believed to be specifically distinct but, so far, I do not think anyone has come to a definite conclusion regarding any of these bodies though they are apparently undoubtedly parasitic. The same is probably true of the small spherical pink-staining (with Giemsa) bodies found in the red cells of the leather-jacket fish of the Australian seas. Multiple infection of the red cells was, however, never present, a point rather against their being protozoa.

Free spores of *Sarcosporidia* in the blood

Another point to be borne in mind is that Probat of Muansa and Francis of Texas have found what seem to be the free spores of *Sarcosporidia* in the blood of animals.

These few notes on the less known human and animal blood parasites conclude this paper. No one is better aware than I am how imperfect and incomplete it is; but, for the most part, it has been written as the outcome of practical experience, and if, with the aid of the illustrations, it saves some from certain pitfalls into which the writer has fallen, and helps others over the hæmatological stiles which bar the way to progress, it will have served its purpose and not have been penned in vain.

It has not been thought necessary, in a paper of this kind, to give references in detail, as the article itself is intended, in some measure, to obviate the necessity of frequent recourse to books and periodicals.

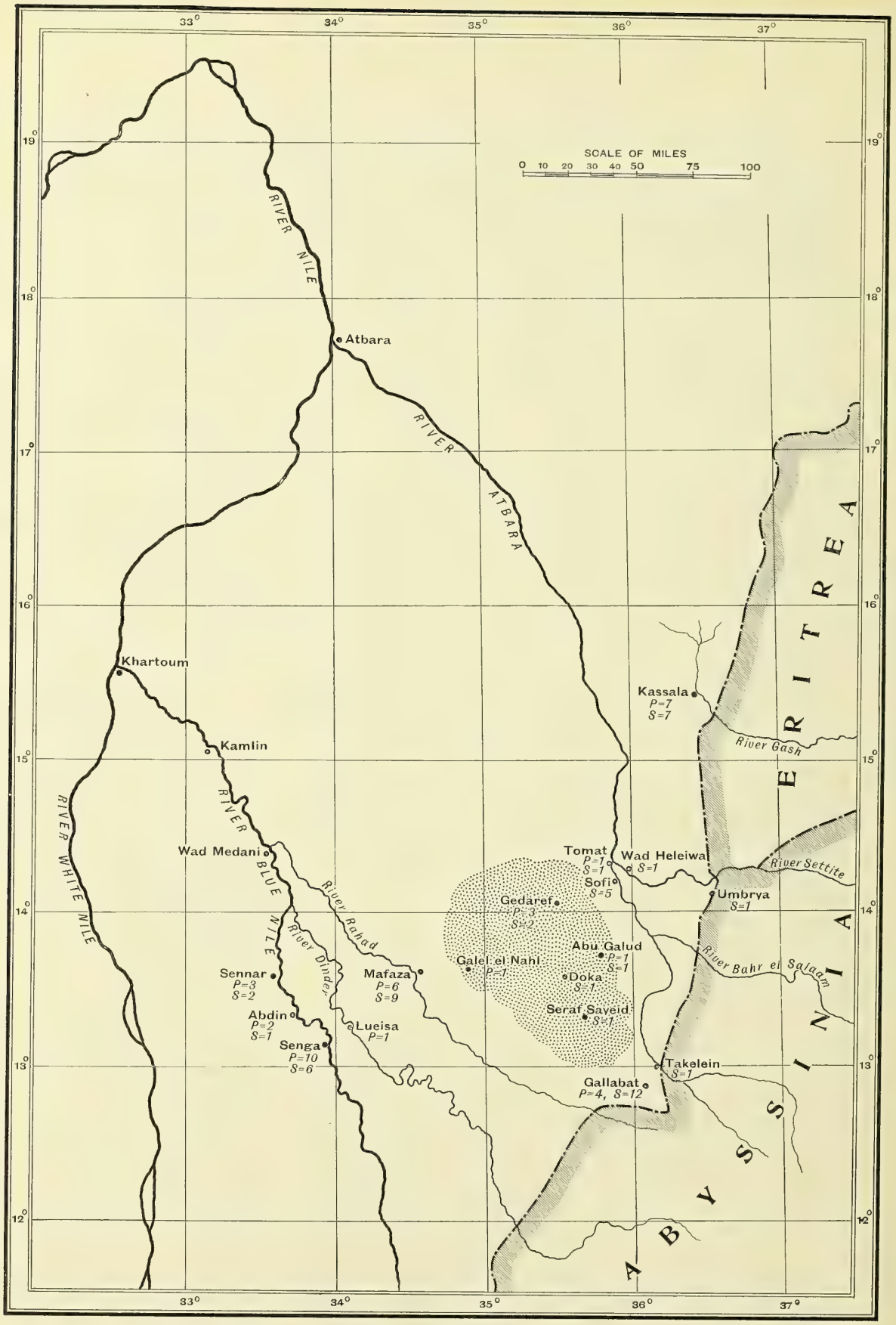


FIG. 31.—ROUGH MAP, SHOWING DISTRIBUTION OF KALA-AZAR IN KASSALA AND BLUE NILE DISTRICTS UP TO MAY, 1909

The Sudan area (dotted tint) may be considered to be practically free, except from imported cases

P = Definitely proved cases *S* = Suspected cases

To these cases must be added—

(1) From White Nile (one) (2) Late Dr. Pirrie (3) From Keili (one) (4) From Singa (one)

Remarks on Kala-azar in the Kassala and Blue Nile Districts of the Sudan

by

Captain L. Bousfield, M.A., M.D. (Cantab.), M.R.C.S., L.R.C.P., R.A.M.C.

Late attached Egyptian Army

The Sudan Government caused an investigation to be made with the object of finding out if kala-azar existed or was prevalent in the above-named districts in 1909. A report was submitted to the Government in May of that year, and this article, at Dr. Andrew Balfour's request, deals with the main points in that report.

Extent of tour

The tour lasted from the middle of January to the middle of May, 1909, and, during that period, some 1,300 miles had to be covered, 900 of which were by camel, and, since more than half of the available working time had to be spent on general medical work, the actual time at my disposal for this investigation was quite inadequate for anything further than a rough estimation of the amount of the disease present in the various communities visited.

Those who may wish to see a fuller account are referred to the August and September numbers of the *Journal of the Royal Army Medical Corps*, 1910.

The accompanying table shows the towns and villages visited and the number of cases, divided into three classes :

(1) Proved microscopically.
(2) Clinically certain.
(3) Highly suspicious.

Only those in whom definite parasites were found are included in Class 1.

The table shows that 57 proved and suspected cases were seen and points to the Blue Nile being seriously infected, for only three places were visited, viz., Sennar, Abdin and Singa, and yet ten cases were found in them.

When the report was written, 42 cases of proved kala-azar had been found in the Sudan, and of these, 15 at least had contracted the disease on the Blue Nile.

Of these 42 cases, 41 have been diagnosed since May, 1907, and have contracted the disease either on the Blue Nile or in the Kassala District.

The fact that the disease has been found mainly amongst government officials and employees calls for attention, when one remembers that they are far more under the medical eye than are the natives.

Distribution along river banks

The map shows the distribution of the cases proved and suspected, and an attempt has been made to piece together all available information, so that the map is a composite one showing all known cases in these stations and does not represent the numbers seen on this tour; since its compilation, fresh cases have come to light.

There is fair evidence to support the belief that all the cases shown in the Sudan area (dotted tint) were imported.

Thus it seems probable that, as in India, the disease tends to cling to river banks, but it is to be noted that in none of these places off the rivers was I able to stay longer

than two days, a time totally insufficient to gain a very reliable knowledge as to the existence of cases. However, it can be definitely stated that the disease exists to no large extent in these towns and villages at present. Visits extending to a week or more were made only at Gallabat, Mafaza, Singa, and Sennar.

Province	Place	Number of patients treated	Kala-azar			Total, proved and suspected
			Proved	Clinically certain	Highly suspicious	
Kassala ...	Kassala	Hospital	1	2	3	6
	Tomat	12	1	—	—	1
	Sofi	12	—	2	1	3
	Wad Heleiwa	7	—	1	—	1
	Gedaref	Hospital	3	—	2	5
	Shesheina	19	—	—	—	0
	Abu Galud	21	1	1	—	2
	Seraf Sayeid	12	—	—	1	1
	Gallabat	161	3	4	3	10
	Tukelein	4	—	—	1	1
	Doka	5	—	1	—	1
	Kom Shetta	14	—	—	—	0
	Matna	9	—	—	—	0
	Galel-el-Nahl	7	1	—	—	1
	Wad Shusha	None could be induced to attend for treatment				
	Mafaza	152	1	2	2	5
	Keili[1]	—	1	—	—	1
	Lueisa	9	1	—	—	1
Blue Nile	Singa	Hospital	5	3	2	10
	Abdin	25	2	1	—	3
	Sennar	152	3	0	2	5
White Nile ...	Costi	6	—	—	—	0
	Total ...	627	23	17	17	57

In no place was the disease in any way evident, and cases had to be found by careful enquiry and examination. The following facts are cited to show that in some cases the disease is epidemic to a limited degree even at the present time, though in the vast majority of cases I could only find single individuals attacked, the other members of the family or community showing no signs of the disease.

(1) At Sennar two servants of an official were found very ill with the disease, one died while I was there and the other was apparently dying. Further enquiries elicited the report that two other servants had died in Khartoum after a good deal of fever in 1908,

[1] Case found on return in Khartoum Military Hospital.

both having greatly enlarged spleens; proof of their deaths in Khartoum I was not able to obtain. The marmiton (cook's boy) also died of fever in the same year. All apparently occupied the same quarters.

(2) At Mafaza, between August, 1907, and April, 1909, amongst those occupying the police lines, eight died of this disease, while another policeman, the sub-mamour and his two servants almost certainly died from this cause.

Associated cases

(3) At Abdin, two brothers were found, one with definite kala-azar, while the other had been ill for two years with fever and had a spleen reaching to the umbilicus.

There was practically no time to study the important question as to how the disease is transmitted, but the following facts were forcibly impressed upon one.

(*a*) Bed-bugs are extremely common; quite 75 per cent. of the native angareebs harbour them;

(*b*) Angareebs in places away from the river banks seem to harbour bugs just as frequently as those in places situated on the rivers.

The following facts, with reference to the presence or absence of bugs in angareebs, were ascertained in 20 cases of kala-azar:—

(1) Live bed-bugs found in eight instances;

(2) Recently deposited eggs, but no live bed-bugs found in four instances;

(3) No signs of bugs or their eggs in four instances;

(4) In the case of those who did not use angareebs, or had not used them in the place where seen, bed-bugs were found in four instances.

All the bugs examined were believed to be *Cimex lectularius*, owing to their presenting distinctly flattened or concave edges on the dorsal aspect of the pro-thorax.

Cimex rotundatus has, I believe, at present never been identified in the Sudan.[1] The disease in the Sudan certainly seems to spread, in some cases, through the members of a household or community, and this suggests that it may be conveyed by the bed-bug. Against this view, however, is the striking fact that in the Sudan, as in India, the disease appears to cling to river banks, and yet bed-bugs are equally common in places on and away from the river and infected persons must frequently be visiting these places off the river.

Bed-bugs

The popular belief, which I have often heard expressed, that angareebs strung with strips of hide do not harbour bugs is quite fallacious, as many such angareebs were seen teeming with these pests. In view of the possibility of the disease being carried from domestic animals to human beings, the animals kept in the *tukls* or *hooshes* of those suffering from kala-azar were noted, but in no instance were animals, evidently ill, discovered. The domestic animals seen were donkeys, sheep, goats, dogs and chickens.

Trade, habits, and food seemed in no way connected with the transmission of the disease, but it still has to be determined whether river water, or the eating of fish, may not be a means of conveying the disease in the Sudan, a possibility in view of the tendency of the disease to cling to river-side villages and the frequent initial intestinal disturbance.

The disease runs in many cases a very severe and rapid course, and of those diagnosed on this tour, eight were known to be already dead in June, 1909. The average duration of the illness, according to the statements of the various patients, who, however, are not by any means entirely to be relied upon, was twenty-one-and-a-half weeks, while five terminated fatally in thirteen weeks. Nine cases gave a history of illness of less than three months, while five more stated the disease had lasted five months or less. The most rapid case was that of a woman aged about 18, at Kassala; she and her husband were

Clinical type often severe and rapid

[1] It has now been found in the Red Sea Province and the Lado district.—A.B.

most definite in their assertions that she had been ill only twenty days before admission to hospital where she died six days later.

Another case, a boy at Abdin, declared most definitely he had only been ill 15 days, and had not previously had fever. He was evidently very ill indeed, but probably the history was faulty as his spleen nearly reached the umbilicus; his peripheral blood showed Leishman parasites.

Chronic cases were seldom seen; three, however, gave histories of five years (Lueisa), three years (Abdin), and two years (Abu Galud) respectively.

Is the disease a recent introduction into the Sudan?

The following points make one inclined to the view that the disease may be a new arrival amongst a previously uninfected people: (1) The virulent character; (2) the comparatively few chronic cases observed (this, in my opinion, is a very suggestive fact); (3) the epidemic character the disease has assumed at Mafaza and Sennar; (4) the absence of history of a past epidemic, though this is naturally extremely difficult to determine owing to the ever-present malaria after the rainy season both near and away from the rivers; (5) the failure of medical recognition, though it must be kept in mind that the disease has been but comparatively recently separated from malaria and many medical men are still ignorant about this malady.

No information was obtained as to the incubation period of the disease, though much attention was paid to this point.

The only case that in any way threw light on this point suggested 16–18 days as the incubation period.

The incidence of the disease appears to favour the months of July, December, and January, which correspond to the commencement of the cool and rainy season and the few months following the end of that season. It is often quite impossible to obtain definite information from a native about the date of onset, but the following table gives the approximate months of onset of all the cases, proved or suspected, that I have been able to follow:—

Incidence by months

KALA-AZAR CASES

Month	Proved	Suspected	Total
January	5	3	8
February	2	3	5
March	1	2	3
April	1	2	3
May	—	—	—
June	—	1	1
July	6	—	6
August	—	5	5
September	2	—	2
October	1	—	1
November	—	7	7
December	5	—	5

A point to be carefully remembered in the Sudan is that it is quite common for a kala-azar patient to have had malaria and to give a history of fever every alternate

day, probably recognising the malarial paroxysms while failing to recognise the fever on the intermediate days.

The average age of those affected was about 18 years, the eldest being about 40 and the youngest about 6. These figures are only approximate, as the ages had of course to be judged on appearance and medical examination. Age, sex and race

Nineteen out of the twenty-two were males, the three females being extremely ill, and, in fact, manifestly moribund, when seen.

This great preponderance of males over females only illustrates the difficulty a doctor experiences in seeing the female population of a Mohammedan community; in all probability the two sexes are attacked equally.

The attached table gives the nationality:—

Nationality	Kala-azar	Suspected Kala-azar	Total
Arab	13	20	33
Sudani	5	1	6
Mixed origin	2	8	10
Egyptian	2	1	3
Abyssinian	—	3	3
Turk	—	2	2

It is also worthy of note that four Englishmen have been reported as contracting the disease in the Sudan.

The preponderance of Arabs is but natural, considering that they greatly exceed the other types in number in the districts visited.

From a study of the death-rate statistics in the main stations visited it would appear that the death-rate is rising considerably in Singa, Gallabat and Mafaza, especially in the first-named town. The fallacies are that the populations of these towns vary greatly, often year by year, and, further, every year probably sees an improvement in registration.

The actual figures for Singa are for 1907, 224; for 1908, 361; and till the end of March 1909 (*i.e.* 3 months), 109.

I believe the death-rate at Singa is increasing in a greater ratio than is the population; it is to be noted that 109 deaths were registered up to the end of March, and if this be kept up for the three remaining quarters, then the total for 1909 will be 436. The death-rate is usually greater in the latter half—*i.e.* during the rains and subsequent fever period—than in the first half of the year, so that probably this total will be exceeded and the death-rate will have *more than doubled in three years.* Death-rate

The onset was either by short attacks of fever, frequently repeated, or by a serious continuous attack.

Personal observations on the type of fever were of too short duration to be of any value, but it was noted that the chronic cases seldom had any fever or only a rise to 99° F. at night; most of the cases seen within a few days of death had a normal or subnormal temperature.

Wasting was marked, especially in the chronic and very acute cases (*vide* Figs. 32, 33a, and 34). Many of the subacute type showed but slight signs of emaciation, and this renders the diagnosis more difficult, since it is not easy to determine whether some Arabs,

who are naturally thin, are wasted, especially as many are poor and therefore badly fed. Emaciation was marked in eleven cases, slight in eight, and not evident in three.

Weakness was noticeable in all cases, but especially in the very acute, while in the subacute it was not very striking.

Protuberance of the Abdomen. In thirteen out of the twenty-two cases the abdomen was *not* prominent, while in several cases it was actually the reverse. Those who had large abdomens were the chronic cases, and the average duration of these cases, when seen, worked out to nineteen months.

Clinical characters

The fact that 59 per cent. showed no protuberance is, to my mind, highly suggestive of a new disease, likely to take on an epidemic character. No chronic case was seen without some protuberance of the abdomen, a condition probably essential, and due to nature making accommodation for the greatly enlarged spleen and the enlarged liver.

Enlargement of the Spleen. In nineteen cases out of the twenty-two the spleen was enlarged to within an inch of the umbilicus or to a greater extent. In the remaining three the spleen was palpable or extended one inch below the costal margin. In all cases the enlargement was regular, the edge definite; tenderness on palpation was complained of in three cases.

Very large spleens were always and only found in chronic cases; the very acute having but comparatively slight enlargement of this organ and no prominent abdomen.

Enlargement of the Liver. In seventeen out of the twenty-two cases the liver was distinctly enlarged though in most cases to no great degree. The largest extended downwards to the level of the umbilicus and the liver dulness was increased upwards one costal space. In no case was it noticed to be smaller than normal.

Jaundice was found in two cases, but in neither to a marked degree.

The conjunctivæ were distinctly of a yellowish tinge in seven of the twenty-two cases.

Pigmentation was only once seen, and then it was doubtful if it was due to kala-azar, and was confined to a curious bronzing of the face. There were no special changes noted in the skin, hair, or nails, and the nervous system appeared normal in most cases.

Mental depression was very marked in four cases, but all were acutely ill; one case was extremely deaf (no quinine had been taken), the power of speech was greatly impaired, and there was marked mental dulness for three weeks before death. No malignant tertian parasites were found in this case.

Diarrhœa was a very marked and serious complication in twelve out of the twenty-two cases. It was often, according to accounts, accompanied by the passage of blood and slime in the stools.

Undoubtedly several cases started their disease with attacks of diarrhœa, and my belief is that the majority had this complication early in the course of the disease.

The frequency of diarrhœa, accompanied by the passage of blood and slime in the stools, naturally suggests the possibility of infection through the alimentary tract, and is a point that should be carefully investigated in the Sudan.

Œdema of the legs was noted five times, one patient presenting œdema of legs, face and a slight effusion into the peritoneal cavity, while another showed œdema of legs and face. In the few cases where the urine was examined no trace of albumin or bile could be found.

Absence of suppurative processes in the cases seen

Suppurative processes were practically absent. Only one proved case showed some boils scattered over his legs, and one suspicious case had a suppurative condition of the scalp; this is no greater percentage than would be found amongst the general population.

Pain was never a marked symptom, though several complained of attacks of pain over the splenic region, these attacks apparently lasting several days and due, possibly, to sudden enlargement of the organ or to adhesions.

Headache, pains in the lumbar region and in the shins were sometimes encountered, but invariably accompanied by fairly high fever.

Epistaxis was troublesome in one case, and bleeding gums were encountered on several occasions.

Hæmoptysis was complained of by one patient, who showed no physical signs of phthisis, and there was no available sputum for examination when the patient was seen. Impossibility of performing post mortems

It is much to be regretted that no post mortem examinations were made, but this was next to impossible owing to there being no available place, the *tukl* being usually in a compound occupied by many other people, and the prejudice against such a procedure very strong.

Figs. 32-35 (*page* 135) illustrate various types of the disease as met with in the Sudan :—

1. A subacute case.
2. Comparison between chronic malarial and kala-azar patients.
3. Extremely acute case of kala-azar.
4. A doubtful chronic case of "kala-azar."

Owing to the very short time allotted, it was considered advisable to make a definite diagnosis by splenic puncture. The writer is well aware this procedure is open to criticism, but circumstances must be taken into consideration, and as patients were usually seen but once, even if blood-counts, etc., had been made, the diagnosis would still have been in doubt, and the slight risk to the patient had to be faced rather than leave a case at large to infect the general community. Altogether I have now performed over 120 splenic punctures without any dangerous symptoms or bad results. Calcium chloride was never administered and many cases walked away 15 to 30 minutes after puncture. Splenic puncture

My experience of liver puncture, a procedure held to be safer, is small, and in the few cases in which I have employed it, I have been unable to find parasites. The cases had but slight splenic enlargement and therefore liver puncture was undertaken, but they may not have been examples of kala-azar.

When patients are under observation, probably liver puncture should be first employed, and, if negative, splenic puncture undertaken. I have met several instances where doctors have used a small exploring needle and syringe for splenic puncture, and to my mind this is but courting disaster, and quite unnecessary.

Recently in Egypt, a doctor told me of two fatal cases, one from splenic and the other from liver puncture, but in these two cases such a syringe and needle had been employed. Only three of my cases showed any symptoms after puncture and these were trivial :— Dangers of faulty technique

1. One case vomited and fainted ten minutes after puncture, but rapidly recovered and had no further unfavourable symptoms.

2. A case, whose maximum temperature had been 100° F., had a rise the evening after the puncture to 105° F., but then reverted to his former type of fever.

3. One case had pain for twenty-four hours over the site of puncture, but no accompanying signs.

I have never seen any signs or symptoms pointing to blood effusion into the peritoneal cavity.

The method recommended for puncture

The method employed for splenic puncture was careful cleansing of skin, hands, and sterilisation of an all-glass hypodermic syringe, with a *hypodermic* needle about $1\frac{1}{2}$ inches long.

The site of puncture was carefully located before the little operation. The patient was told to take a deep breath and hold it; several preliminary exercises were carried out, so that he fully understood what was required and that he was not to let go his breath till the needle was withdrawn. The needle was then inserted rapidly and at right angles to the spleen, and several drops of blood were at once drawn off. The proceeding did not cause pain to any degree, and the whole performance was finished in about five seconds or less.

In some cases the lax and thin abdominal wall allows of the spleen being more or less fixed against the lower ribs by inserting the hand under its edge and pressing firmly upwards and outwards, and in these cases inspiration need not be employed. The cases where danger may occur are those who are very nervous or very young; my small experience does not seem to point to advanced anæmia being a cause of danger; many patients punctured were extremely anæmic. In such cases, should sudden expiration take place, it is of the utmost importance, I believe, to hold the syringe very loosely, so that the movement of the syringe in the direction of the long axis of the spleen is in no way hindered, and thus the needle with the spleen is not fixed; if held firmly and fixed, the needle might be very liable to cause a rupture of the splenic capsule.

It was found that if the syringe rapidly filled with blood, the chances of finding parasites were small, and the blood usually had more or less the characteristics of peripheral blood; probably in these cases the blood came directly from a splenic sinus.

In a few cases it was found almost impossible to draw off any blood, in one case even after three punctures; usually, however, enough was obtained, though not sufficient to make a good film. In these cases it was noticed that the spleens were not easy to puncture, and the difficulty was probably due to a thickened capsule. By giving a few slight lateral movements to the needle the splenic pulp is slightly damaged, and blood can, in some cases, be drawn off, as in puncture of a lymphatic gland, though such a procedure should be avoided unless absolutely necessary.

A positive spleen puncture settles the diagnosis, but a negative result still leaves one in doubt.

Appearance of the blood withdrawn

In three definite cases, and in several that were clinically kala-azar, the blood withdrawn had, to the naked eye, the appearance of slightly clouded serum, though undoubtedly coming from within the spleen itself; this was noted especially in severe and rapid cases of kala-azar.

The method employed for splenic puncture has been given in some detail, but it must be kept in mind that this method was used with natives, who are not highly strung, and either do not show or do not feel pain as the civilised white races do. Hence the method of taking a deep breath and holding it, by means of which the spleen is depressed and held downwards towards the pubis, might quite well with a hyper-sensitive white person be most dangerous, as the natural inclination of a person who has not full control of himself would be to let go his breath on puncture, and thus a more serious tear occur in the splenic capsule than if no previous deep inspiration was taken.

In dealing with an individual of a white race, especially if of a nervous temperament, I would suggest that it would be better to produce a reflex cessation of respiration by telling him to take a series of deep breaths and so produce a condition of apnœa for the puncture.



L. Bousfield

FIG. 32.—CASE 1

Boy, aged about 9, at Gallabat. Ill probably six months. Limits of spleen and liver delineated in white paint. Emaciation marked. Died March 27, 1909.



L. Bousfield

"A" FIG. 33.—CASE 2 "B"

Two cases at Singa. "A"—Boy with kala-azar. Ill eleven months. Died May 28, 1909. "B"—Girl from same house, but suffering from profound malarial infection. This photograph shows plainly the difference in wasting and in the prominence of the abdomen.



L. Bousfield

FIG. 34.—CASE 3

A very severe and acute case at Gallabat. Duration three months. Died March 23, 1909. Diagnosed at first as dysentery. Marked wasting, and weakness very pronounced. Enormous number of parasites in spleen blood. Peripheral blood like serum. No malarial parasites found. Spleen not very greatly enlarged.



L. Bousfield

FIG. 35.—CASE 4

Case at Gallabat. An Abyssinian, ill two years. Typical parasites could not be found in spleen blood. No malarial parasites in peripheral blood. Liver and spleen enlarged. Wife ill with fever for five months. Fever in both cases unaffected by quinine.

No cases that presented only atypical bodies were included in the list of those definitely diagnosed, though it is a striking fact that these bodies were *only* found in those with typical parasites, or in cases that were clinically kala-azar. These atypical bodies are illustrated in Plate VIII., figs. 7, 8, 9, 15 and 16, while others were frequently seen having no signs of a blepharoplast.

In ten cases the parasites were readily found, and the diagnosis settled by a few minutes microscopic work, but the remaining twelve needed much laborious work before they could be demonstrated.

Relation of number of parasites to severity of infection

Major Cummins, R.A.M.C., stated that the number of parasites found had no relation to the severity of the infection. My experience certainly does not coincide with his, for my cases, which were acutely ill with the disease, practically invariably presented a large number of parasites in the splenic films, and only required a few minutes for microscopic diagnosis, provided that true splenic blood was withdrawn. In contrast to this, the chronic cases presenting wasting, prominent abdomens, large livers and spleens, and a low fever, and which could easily be diagnosed on clinical grounds alone, presented very few parasites, and often it was extremely tedious to demonstrate them, while in several such cases there was an absolute failure. I do not think any of these were chronic malarial cases.

Fifty-six splenic punctures were performed during this tour, and twenty-two positive results were obtained. In thirty-nine cases, which were undoubtedly kala-azar from a clinical standpoint, nineteen showed parasites in their splenic blood—*i.e.* in about 48·5 per cent. of the clinical cases the parasite was demonstrated.

Other aids to diagnosis, such as the type of fever, the effect of quinine, leucopenia and differential counts, could seldom be employed, owing to the impossibility of following up the cases and to lack of time.

Other aids to diagnosis

Though a differential count is most useful when one has a patient under constant observation, yet for such a rapid tour of investigation the method was found by me of little value, the percentages varying considerably. Without finding the parasite I felt that a diagnosis, founded on not very definite clinical grounds, together with a differential count supposed to indicate the presence of the disease, was scarcely sufficient to justify isolation and destruction of property.

In the definite kala-azar cases the finely granular polymorphonuclear leucocytes were usually present in numbers varying from 30–45 per cent., while the large mononuclears varied from 16–32 per cent.

Malarial parasites were not found in a single case of kala-azar, either in the peripheral or splenic blood, and in the suspected cases only in four instances.

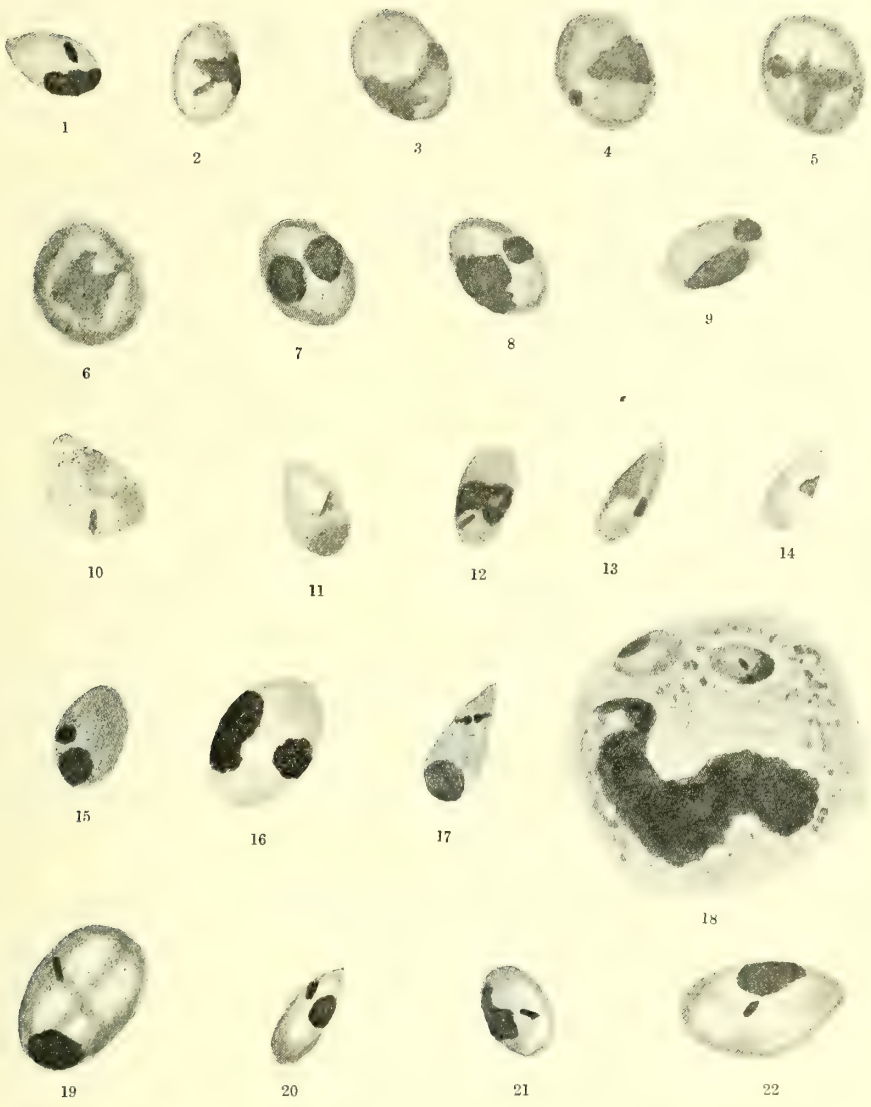
Absence of malarial parasites in kala-azar cases

Considering how extremely common malaria is, this is very remarkable, and I can only suggest that it is a case of the survival of the fittest. The weaklings dying off from malaria in early childhood, those with stronger constitutions surviving and growing up, gain an immunity, so that, although suffering from malaria during the rainy season, yet they are capable of ridding their general circulation of the parasites when once the malarial season is over, in spite of their not taking quinine. This tour was made during the non-malarial season.

In several cases the peripheral blood was watery and spread on the slides extremely badly. In three virulent cases it resembled cloudy serum.

The type of parasite usually encountered was the typical one now so well known as to need no description, Plate VIII., fig. 1. Most of the forms were free and well developed.

PLATE VIII



L. Bousfield

KALA-AZAR

1—6. Parasites found in chronic case (3 years' duration): 1. Typical parasite; 2. Slightly degenerate enlarged parasite; 3—6 are believed to be further stages in the degeneration of the parasites within the body.

7—9. Forms only seen in chronic cases—apparently parasites in which the blepharoplast resembles in size and staining the macronucleus, unless it be a divided macronucleus.

10—11. Degenerate forms found in very chronic case (5 years' duration).

12—14. Degenerate forms found in case (? 6 months' duration).

These forms were found frequently in long-standing apyrexial cases, which presented typical symptoms and signs of kala-azar, and are believed to represent degenerative processes occurring in the parasites within the body.

15—16. Parasites similar to those depicted in figs. 1—14.

17. Parasites showing curious division of blepharoplast.

18. Parasites found in leucocyte in the peripheral blood.

19—22. Leishman parasites, after one injection of orsudan, showing marked vacuolation.

$\frac{1}{12}$ *objective, No.* 8 *ocular*

Types of parasites encountered

In chronic cases it was frequently extremely difficult to demonstrate the parasite, though on several occasions structures were seen which I believe to be degenerated parasites. These are illustrated in Plate VIII., figs. 10 and 11. The cytoplasm in these bodies was often degenerated, staining badly, often taking a reddish tinge and showing granulation and excessive vacuolation; the macronucleus was often ill-defined and faintly stained, the blepharoplast (?) diffuse and staining like the macronucleus.

These, however, appeared to be parasites owing to their definite borders, the protoplasm staining blue and containing a chromatin mass, and what appeared to be an altered and degenerate blepharoplast. These changes were never noted in the smaller and younger parasites, nor in those of virulent cases. Excessive vacuolation was seen in parasites after a single injection with orsudan (*vide* Plate VIII., figs. 19-22).

Further, in chronic cases, parasites (?) were found which showed no signs of a blepharoplast as usually seen, but two more or less equal chromatin masses usually equally deeply stained—sometimes one, usually the smaller, somewhat more deeply coloured (*vide* Plate VIII., figs. 7, 8, 9, 15 and 16). Such structures were only seen in kala-azar cases, or those clinically like this disease. These forms did not appear degenerate like the others already described.

Occasionally parasites were observed of about the diameter of a normal red corpuscle, sometimes quite circular in outline, but typical in the possession of nucleus and blepharoplast. The cytoplasm, however, was more granular and vacuolated than in the younger oval forms. One parasite seemed to be within a red cell and appeared to be exactly in focus with the edge of the corpuscle, but since this was the only one seen in a very large number of films, it is probable that it was superimposed. Parasites within cells were not seen with any great frequency, possibly due to the fine type of needle used.

In only two cases (both acute) were parasites found in the peripheral blood, in one case free, and in the other free and in phagocytes; no attempt was made to find parasites in centrifugalised blood.

Occasionally a parasite with a bi-partite blepharoplast was observed; in one case the adjacent ends of this divided micronucleus were distinctly enlarged (*vide* Plate VIII., fig. 17).

Smears from the blood and various organs of the "Urmoot" fish were examined with negative result; this fish is largely eaten at Mafaza. Bed-bugs (*Cimex lectularius*) were dissected on several occasions and smears made from various parts but with no definite results—considerable previous practice and experience is required for this work, and without such knowledge and experience the observations are not trustworthy.

I can only say that two structures, very like kala-azar parasites, were observed in a smear from the mid-gut of a bed-bug, gorged with blood, which I had caught on the angareeb (bed) of a very virulent case of kala-azar.

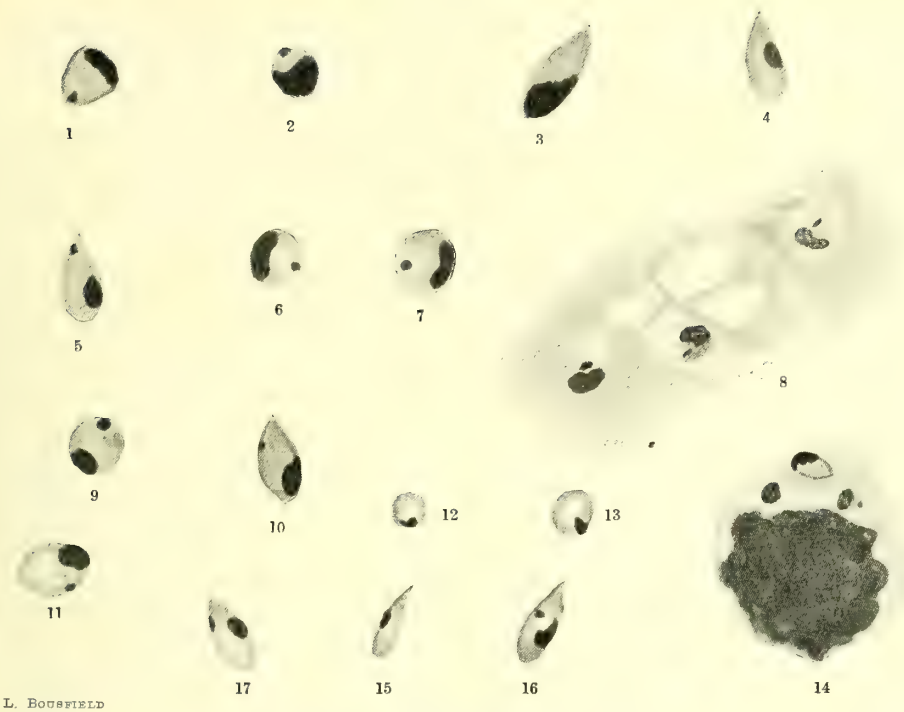
Examination of dogs

Two dogs were killed and smears made immediately from the spleen, liver, kidneys, mesenteric glands, and heart's blood—

1. A dog, male, about 4 years' old, belonging to an extremely acute case of kala-azar and living in the same *tukl* with the patient, was killed and examined at Gallabat. It was very well nourished and exceptionally clean for a native dog. No *ecto-parasites* were found.

Nothing abnormal was noted in the organs except that the mesenteric glands in connection with the small intestines were greatly enlarged and of a deep maroon colour, while the two largest appeared lighter in colour, and as though they were about to break down and suppurate. The intestines connected with the glands showed no macroscopic disease.

PLATE IX



L. Bousfield

DOUBTFUL "BODIES" FROM A DOG

Structures, possibly kala-azar parasites, found in an apparently perfectly healthy dog which lived in a compound containing a very virulent kala-azar case, a woman aged about 22, at Gallabat.

1—8. Structures found in smears from spleen.

9—11. From liver smears.

12—16. From mesenteric glands, which were much enlarged.

17. Only structure in any way resembling a Leishman-Donovan body found in the kidney smears.



L. Bousfield

UNRECOGNISED BODIES IN KALA-AZAR SPLEEN FILMS

Structures found in spleen blood from case at Tukelein, man aged about 35.

18. Normal red cell.

19—25. ? Parasites. No intra-corpuscular forms found. Distributed about the slides, but usually more or less aggregated together.

24—25. Found lying together as drawn. Short detailed account of case given.

Smears were made from the spleen, liver, kidneys, and mesenteric glands, and a few structures were found which were extremely like Leishman-Donovan bodies (*vide* Plate IX., figs. 1-17).

It is impossible to say definitely what these are, as films only were made, and it was quite impossible to attempt any cultures.

Very few bodies, scattered here and there, could be found in the films, and in the kidney films only one was found after prolonged search.

All that can be said is that these structures have a striking resemblance to kala-azar parasites, and work should be carried out on dogs in the Sudan.

2. The second dog, though very wasted, showed no structures similar to the Leishman bodies, but many filariæ were found in the liver and spleen smears; this dog was a vagrant.

I wish to thank all who most kindly assisted me in my investigations, and to record that every help and courtesy was afforded me by the English and Egyptian officials and the medical officers, amongst whom I would specially thank El Sagh, Mohammed Eff, Ali Niklawi, Sudan Medical Department, who gave me much assistance and information and kindly sent me several detailed reports on the cases at Singa.

Recommendations for prevention

The following is a brief summary of the recommendations suggested to combat the disease :—

1. Burning of infected huts, angareebs, etc.

2. Isolation of acute cases, and compulsory isolation villages for chronic cases.

3. Special quarters to be allotted to Abyssinians in towns and villages.

4. Careful medical examination of Abyssinians before enlistment into the army or police, or before employment as servants to officials.

5. That all officials in the infected districts who have to visit villages be supplied with tents, and that all possess small camp-chairs.

6. Avoidance of native angareebs by officials, their servants and police.

7. A course of instruction to officers of the Military and Civil Medical Departments on this disease, especially with regard to its pathology and microscopical diagnosis.

8. Until our knowledge of the transmission of the disease is more definite, it would be wise for officials not to keep dogs.

9. That all the angareebs used by the soldiers and police be placed, uncovered, exposed to the sun from early morn to sundown. My belief is that one day's exposure to the tropical sun of summer would destroy all the bugs, even those within the crevices.

This requires a series of experiments; in the one instance in which I tried it I could find no live bugs after one day's exposure, though there were many to be found previously.

10. The invaliding from the army and police of those who are weak and wasted and have enlarged livers and spleens, with subsequent supervision.

13. The most effective way of getting rid of bugs from woodwork and crevices in walls, etc., is to play the flame from a painter's lamp direct, and subsequently apply a thick wash of lime.

14. Further investigation of the disease, especially up the Blue Nile.

This has already been done by the Second Commission sent by the Sudan Government in 1909, and its report will no doubt add very greatly to our knowledge of the extent, distribution, pathology, etc., of this disease in the Sudan.

That the acute epidemic type as well as the sporadic type exists in the Sudan is now well established, and several of the most acute virulent cases did not raise in my mind the

diagnosis of kala-azar but that of an acute fever; several times that of dysentery. It was a great surprise, therefore, to find the enormous numbers of parasites procured from the puncture of the but slightly enlarged spleen.

These acute cases are extremely important, and medical men must keep the possibility of kala-azar in mind in the case of acute fevers in the Sudan—as for chronic cases they are walking advertisements of their disease.

Appendix

Unidentified bodies were found in the splenic blood of a case suspected to be suffering from kala-azar.

The history of the case was as follows:—

An Arab, aged about 35, had lived all his life at Tukelein, near the Atbara, near Gallabat. He had been ill two or three months with cough and stated he had at times expectorated blood. He was very thin and wasted. There was no other history of illness and he stated he had never had fever, but when seen at 10 a.m. his temperature was 100·2° F.

Family history.—His wife had died three months previously after three months continuous fever. She had no dysentery, cough or expectoration of blood. His boy, aged about 5, was said to be quite well, but on examination both the liver and spleen were found to be considerably enlarged and his temperature was 100·1° F. The father and the child himself said he had not been ill with fever. The lungs and heart appeared normal.

Unidentified bodies found in spleen of "suspected" case

Physical examination.—Marked wasting and general weakness. Patient evidently seriously ill. Slightly anæmic. Conjunctivæ not yellow. No œdema. No physical signs of lung or heart disease. Spleen enlarged one inch below the costal margin. Liver not enlarged. A splenic puncture was performed, but very little blood could be withdrawn and the films were made with difficulty. Microscopical examination revealed neither kala-azar nor malarial parasites, but some curious bodies were discovered which are drawn in Plate IX., figs. 19–25. The films were bad, many red cells were distorted, but in those which were intact no intra-corpuscular bodies could be found. It is greatly to be regretted the case was only seen once and no peripheral blood taken. Further, the films were not examined till the next day, as my microscopical apparatus had to be left behind owing to the difficult and stony track from Gallabat to Tukelein.

Possibly these structures are hæmogregarines. The average length was 3μ to 5μ, breadth about $0{\cdot}5\mu$. The central portion was nearly always narrower than the extremities, which were rounded. The outline was definite, the protoplasm stained blue and contained a nucleus which usually extended completely across the structure. No chromatin dots were noted scattered about in the protoplasm and there was no pigment. No trace of an enclosing red cell could be seen.

Possibly Plate IX., figs. 24 and 25 represent vermicules fixed during motion.

Fig. 20 shows a double nucleus, situated in the centre, the smaller lying against the convex border of the parasite in its long axis.

Fig. 23 shows forms very similar in shape to the diplococcus of pneumonia, having blue bodies with definite chromatin transverse bars.

It seems worth reporting this case and drawing the structures, and though it is scarcely possible to say definitely what they are, yet they appear from their definite borders, staining properties, etc., to be parasites. They are scattered here and there throughout the two films taken.

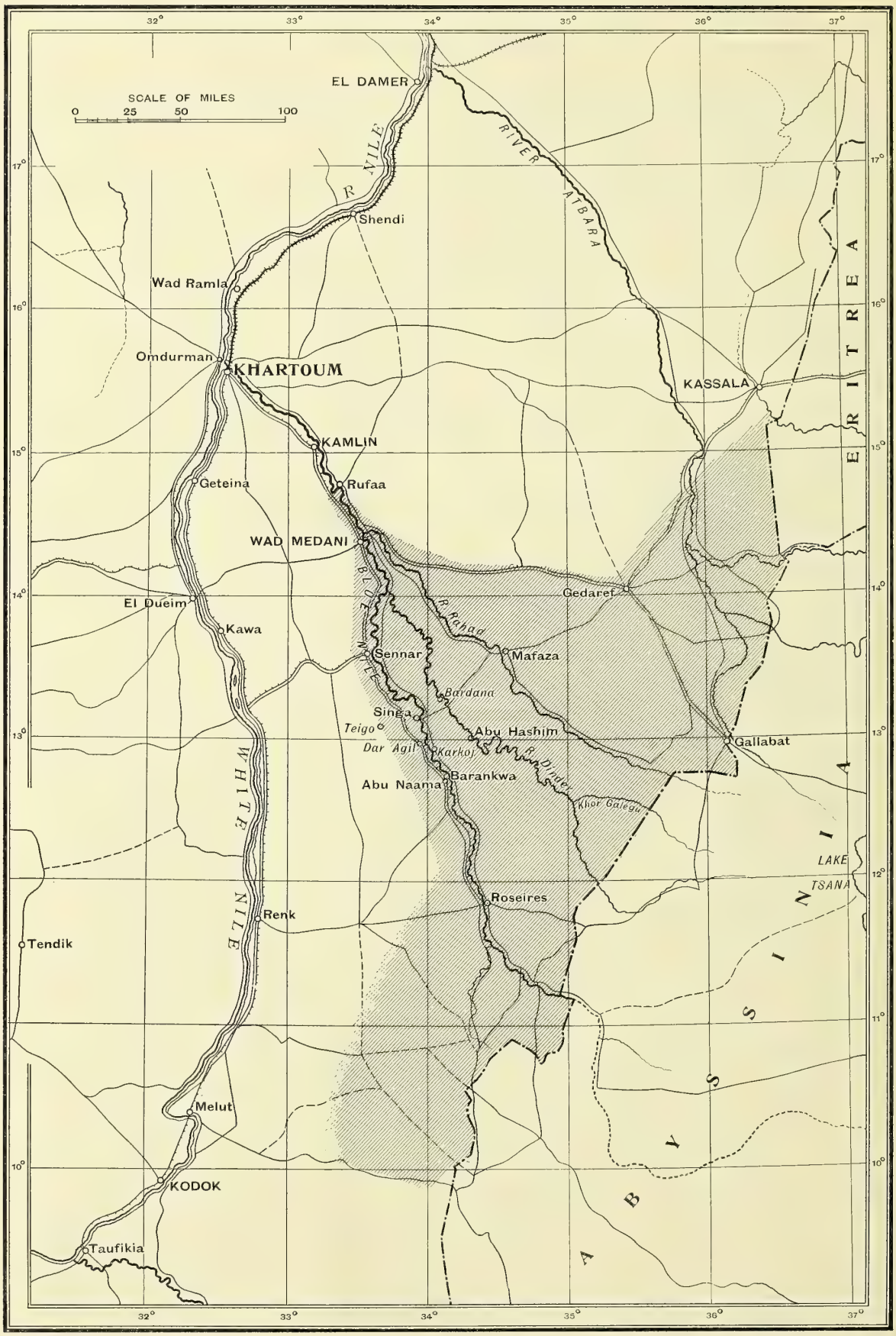


FIG. 36.—MAP OF EASTERN SUDAN SHOWING AREA KNOWN AT PRESENT TO BE INFECTED WITH KALA-AZAR

Kala-azar Commission

to investigate the Prevalence and Cause of the Disease in the Eastern Sudan

(1) General Report

by

Captain D. S. B. Thomson, M.B., B.Ch., B.A.O., D.P.H., R.A.M.C.

Attached Egyptian Army

The Commission, which was appointed by the Sudan Government, left Khartoum on November 3, 1909, on board the steamer *Culex*, with which was the Wellcome Tropical Research Floating Laboratory. Both of these were kindly placed at our disposal by the Director of the Wellcome Tropical Research Laboratories, Khartoum.

It was decided that one of us, the pathologist, should remain in Singa with the Laboratory, the other travelling through the country to determine the area of prevalence of the disease, and if possible its cause.

Tours of inspection

On arrival at Singa, it was decided to make a series of tours of inspection, each taking about two months.

The first, from Singa to Roseires, going south by the west bank and returning north by the east bank.

The second, from Singa to Gedaref, thence to Gallabat, visiting the surrounding villages and returning to Gedaref and Singa by different roads where possible.

The third tour, south to Karkoj, thence east to Khor Agaliin and thence south along the west bank of River Dinder as far as the Khor Galegu, returning north by the east bank visiting all the villages as far north as Bardana, and returning to Singa.

The fourth tour, north to Sennar by the west bank, returning to Singa by the east bank.

The fifth tour, south-west from Singa to Dar Agil, thence north along Khor Um Esh to Teigo and thence north-east still along the Khor to Hillet Howeires and back to Singa.

These five tours took eight months of actual travelling, the last one extending into the month of July, after which, owing to the rains having started and the presence of "fly," camels could no longer be employed.

In all 219 villages were visited and 2,100 miles covered by camel and river.

The time spent in each village varied according to its size. In the larger villages three days were necessary, whereas in the smaller, one day was sufficient to see all the inhabitants and sick. As far as possible a stop of at least one day was made in each place.

Naturally, general medical work was predominant and no difficulty was found in getting the natives to come for treatment. No accurate record was kept of the number of cases treated, but at the lowest computation at least 3000 men, women and children were treated or given advice.

Procedure in villages

On arriving in a village the procedure was as follows:—

The Sheikh was told to warn the people of the English doctor's presence, his willingness to see all sick, and visit in their houses those unable to come to him, and that all medicine, etc.,

was free of charge. One is in most cases met immediately with the reply "Qullina shudad, bi Amanillah" (We are all well, God be praised), but a walk through the village and conversation with the people soon overcomes their fear of the Tourk. One finds that all the maimed, the halt, and the blind, are first sent to see what effect the "Dawa el tourkowi" will have. If one succeeds in a simple case in giving immediate relief, the whole village, who previously were "shudad," will come clamouring for medicines. Quinine is known practically everywhere as a cure for fever and is asked for freely. Purgatives, the stronger the better, are also in great request, no medicine being considered thoroughly efficient unless accompanied by thorough purgation.

Nearly all ills are attributed to "Wisaka fi butn" (dirt in the belly) and they consider it can only be removed by this means.

Many cases of both surgical and medical interest were seen, but the rooted objection to coming to hospital for operation or prolonged treatment is so great, in addition to the difficulties of transporting the patient, that one rarely got cases to come to Singa.

Native information on Kala-azar

The first point to be settled was whether the disease was one recognised by the natives or not. By questioning the older inhabitants of villages it appeared that kala-azar had been well known for some considerable time by the names of "Simeih," "Dobaal" or "Marad-el-Seyid," and by western Arabs as "Abu Safar." This was confirmed in the various villages either by asking some of the inhabitants to describe the symptoms of the disease known as "Simeih" or else by describing the symptoms and asking what the disease was called.

The symptoms as described by them are as follows. The disease appeared generally during the "darrad," *i.e.* immediately after the rains, about October. The fever is persistent, the spleen is enlarged, the patient gradually wastes away, though his abdomen remains prominent. They describe the anæmia by saying "El damm asfar" (the blood becomes yellow); shortly before the end, swellings of the feet and face appear. They notice diarrhœa as a frequent complication, also epistaxis, cancrum oris, sudden swellings, cough with dyspnœa and blood-stained sputum, but these latter, more rarely. The disease lasts as a rule from three to six months, it is practically always fatal and appears to attack all ages, though much more frequently children and young adults.

The disease has two forms, acute and chronic, the acute form being almost entirely confined to the younger cases and the few chronic ones to the older adults.

The name "Simeih" is said to be the Baggara one for the disease, whereas "Dobaal" is a name used by some of the natives of the Southern districts of the Blue Nile and Kassala. "Marad-el-Seyid" is a general term applied to the disease; "Abu Safar" being the name given to this disease by the Western Arabs.

The disease was brought from the west by the Baggara in the time of the Mahdi, and, since then, it has always been present, though apparently never in an epidemic form, as never more than two or three cases were in the same village.

It is now less common and some of the older natives say that though they have heard of the disease and know its symptoms, yet they have never seen a case. Others have seen one a long time ago, or heard of a case in another village.

I questioned many old Taaishi men as to what they knew of the disease in the west. They said it was recognised in pre-Mahdi days, though it was never common.

Cause of Disease according to Native Report

The cause of the disease is, according to native reports, but vaguely known.

The following supposed etiological factors may be of interest, and possibly have some bearing on what may ultimately prove to be the real cause of the disease.

Bad feeding is apparently an essential factor in producing it, drinking water from

wells which have been flooded during the rains, using *Weykab* with food, eating *Mandaki*, sleeping on the ground at the beginning of the rains and so inhaling the vapours which arise from the warm moist earth, all these have been given as causes of kala-azar. Some consider the disease highly infectious and won't eat or drink from the same vessels as the patient or sleep in the same house.

Weykab is made as follows :—

Dura stalks are heaped on the ground and burnt, the ashes collected and put into a *Gurra*, in which there is a small hole at the bottom. Water is poured on to this and filters through into a second vessel. The filtrate is light yellow in colour and slightly saline in taste and is used with food instead of salt, when salt is unobtainable or the people too poor to buy it.

Mandaki is a strong-smelling preparation of raw pounded fish, bones included, made into balls and hung up in the *tukl*, pieces being broken off as required. These fish balls are made either from a mud fish called *Garmout*, or else from *Seer* which they say is a young form of the fish known as *Daps*. Both *Weykab* and *Mandaki* are only used by the very poorest people, and, under exceptional circumstances, by others.

All these alleged causes would seem to point to an intestinal infection caused by food or water getting contaminated by soil.

Some years ago in the Dahari of the Gezira west of Wad Medani, I saw several cases of night blindness which were attributed to the excessive use of *Weykab*.

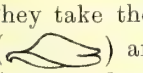
Native Treatment

I wish to thank the Inspector-General, Sir Rudolph Baron von Slatin Pasha, for the following interesting information as regards native treatment which he kindly sent me.[1]

"The disease which the natives of the Northern Districts in the Sudan call 'El Semeih,' is called by the Taaisha Tribes of the West 'Abu Safar,' which means 'father of yellow.'

"It commences with strong fever, the body loses all its flesh and the colour of the skin becomes extremely pale; the abdomen increases considerably in size and the tongue turns white.

"The process of natives in the treatment of this disease is as follows :—

"They take the root of a climbing shrub, called *El Lawia*, whose leaves are of this shape () and that of another shrub called *El Debeigha*, and mix them with 'Iron scale' (*Khara-El-Hadid*), and a handful of red pepper (*Shatta*). All this is pounded into powder. A young he-goat is then slain and skinned; and the head, feet and inside being discarded, the remainder is cut into small pieces and put into a large pot filled with plenty of water. This is mixed with the powder thus prepared, the pot is placed on the fire in the evening and caused to boil for some six hours.

"At daybreak, the patient is first given about ½ a rotl of *Semn* (butter) and then about the same quantity of the prepared broth and a few slices of the boiled bitter goat-meat.

"The effect is that of a very strong purgative, and the same treatment should be repeated for three times in succession (it is advisable to take the broth of this purge a little warm), while at the end of the three days the patient should consume *Semn* in large quantities for another three days, and the diet should consist of meat, bread, milk, but no vegetable such as *Weika*, or what the Sudanese call *El Durraba*."

These shrubs exist in districts south of Sennar, but the natives do not use them.

I questioned some of the older Taaishi on the Blue Nile and they confirmed this, and say that the plant is known here also as *El Lawia*, though the natives of the district were ignorant of its use. So far as I could find out, there is no recognised treatment for the

[1] Statement made in Arabic by "Western Arabs."—R. Slatin

disease on the Blue Nile beyond the regulation native procedure of purgatives, drinks of tannin, etc., cupping, both wet and dry, over the spleen, and blistering or firing, all of which are used in cases of enlarged spleen, and fever.

Infection of Riverain and Inland Villages

During the various tours, both riverain and inland villages were visited. Judging from the number of cases found and reported the disease is unquestionably more common in riverain villages.

Of the various districts visited, beyond doubt the most heavily infected are the Merakiz of Singa and Abu Naama.

Practically all the cases found came from this area. Barankwa was the only place in which more than one case was found.

The villages of Tama and Beheiga may be mentioned as of especial interest.

These villages are situated about 200 yards back from the river on Azaza soil, *i.e.* a light kind of soil, and about 500 yards apart. The ground has a natural slope on all sides and trees are cleared away from the villages for a very considerable distance. In fact, the sites would appear to be ideal ones, yet during the past six years the people have lost so many of their relatives and children, from, they say, "Simeih," that the greater number of them have left the villages, and the remainder wish to go. This has been arranged for. They state that the first year they came there they were healthy and well, but that then the disease began and killed them off, as many as five in the year dying of it.

On the occasion of my first visit I examined all the remaining people of the two villages but could find no trace of the disease in any of them. Nor was I able to get any information about the origin of the disease there, as to whether the first case had been elsewhere, or in contact with an infected patient, or come from a village in which the disease was present.

In the village of Tama the Sheikh reports a case of mother and child being infected and both dying, the mother showing signs of the disease one month after her son's death. This is the only case in which there has been any record of more than one member of a family being infected.

Comparison of number of Cases in Provinces

The comparative figures of cases in various provinces are interesting.

Captain Bousfield, in his report last year, states that of 42 cases reported up to May, 1909, 15 were from the Blue Nile. Of the 41 new cases reported by us during 1909–10, 36 have been seen or reported in Sennar Province.

These figures are very striking, though the fact that the greater part of the time of the Commission was spent in this province, may account for so few cases being noted in Kassala Province, where only six weeks were spent, the reason for this being that it was deemed better to devote the greater part of our time to, as yet, unvisited districts.

Question of Introduction from Abyssinia

The suggestion has been made that the disease is introduced into the Sudan from Abyssinia. The arguments in favour of this as regards Kassala Province are justifiable, though there is no evidence to show that the disease itself exists in Abyssinia. It also might be argued that the habits of the Abyssinians would make them particularly prone to infection in a country in which the disease already exists.

As regards Sennar Province I do not think that the disease can be attributed to Abyssinian sources. The number of Abyssinians coming as far north as, say, Singa is small, and certainly in no town on the Blue Nile is there anything like the colony of these people that one finds in Kassala, Gedaref and Gallabat.

The total number of Abyssinians in Singa is 14, which is a very small percentage of the total population of the chief town of Sennar Province. I think that a great deal more evidence will be required in support of this theory before it can be accepted.

The question of the disease being endemic or epidemic is most important. Epidemic or Endemic?

From all the information obtainable, undoubtedly the weight of evidence is in favour of the disease having been present in the country for many years.

Also it apparently used to be much more prevalent in certain districts than at present, though in no case could I get any information of its occurrence in true epidemic form.

Certainly, in some instances, there would appear to have been small epidemic outbreaks of the disease, *e.g.* the village of Tama. It is difficult to say whether these outbreaks are premonitory of a severe epidemic or merely the last epidemic flicker of a disease which is becoming endemic. In other words, that the epidemic has left behind a sporadic affection.

The latter seems to be the more likely solution under the circumstances.

As regards transmission, the accepted theory, at present, is that the vector is *Cimex rotundatus*, yet this bug has never been found in any of the infected areas of the Sudan. Method of transmission

The native information on the subject would favour an intestinal infection and the possibility of this mode of infection should not be lost sight of. This subject will be fully dealt with in the pathological report (*vide pages* 157–172).

The possibility of infection from animals must also be considered. The *tukls* of natives generally hold at night, all or nearly all the following animals: goats, sheep, hens, pigeons and perhaps a litter of puppies, in addition to three or four human beings. No animals suffering from disease were noticed in the houses of any of the patients.

In several villages I heard of and saw outbreaks of spirochætosis amongst the fowls of the natives. Also outbreaks of *Abnini* had killed off nearly all the goats of some villages.

This is a disease apparently affecting the lungs and is very rapidly fatal. I myself saw no animals actually suffering from this condition.[1]

The procedure on finding a case of kala-azar was as follows:— Procedure on finding case of Kala-azar

The parents of the patient were told that he was suffering from "Simeih," and the advantage of transferring the case to Singa for treatment was pointed out, especially as, according to their views, the patient was bound to die, whereas if sent to Singa there was the possibility of prolonging life. They were told that the *tukl*, together with the *angareeb*, *bursh*, etc., occupied by the patient, would be burnt and ample compensation given for what was destroyed. In no case was there the least difficulty in carrying out this procedure, the parents being only too glad to grasp the possibility of saving their child's life.

When the patient had been removed to hospital, the *tukl*, etc., was burnt under personal supervision and a certificate given to the owner for the compensation, which he drew at the nearest *Markaz*.

Spleen puncture was performed in every case which was at all suspicious and in many others in which splenomegaly was the only symptom. Spleen puncture

No difficulty was met with in making spleen punctures. The skin, having been washed with soap and water, was then thoroughly rubbed with methylated spirits and painted with tincture of iodine. After the needle was withdrawn, a piece of cotton wool soaked in collodium was placed over the puncture.

A 2 c.c. all-glass syringe, with a fine needle, was used in every case. The needle was introduced just below the costal margin in every case but one, in which, owing to the small size of the spleen, the needle was inserted in the intercostal space.

[1] This is infectious pleuro-pneumonia.—A.B.

In some cases, blood did not flow freely until the needle had been withdrawn slightly. The needle was wholly withdrawn as soon as blood appeared beyond its butt, the blood thus obtained being sufficient for many slides.

In all, 53 spleen punctures were done on tour and in no case were any unfavourable results noted. Every case was kept lying down for two hours after spleen puncture, and then carried to his house, and told not to walk about till next day. This order was often not obeyed, and I have met many cases, three hours after being punctured, walking about unconcernedly.

Age incidence

Of the 37 cases seen and reported on the Blue Nile, 24 were in boys and girls from 7 to 18, whereas of the 5 cases noted in Kassala Province, 4 were in men of from 20 to 30 and only 1 in a child.

It would therefore appear as if the type of disease found in the Sudan is very similar to that of India. In India, 50 per cent. of the cases quoted by Rogers were in children and young adults up to 20 years of age. In the report of sporadic cases at Sylhet an even higher percentage, namely 69 per cent., of cases under 20 years, is reported.

This latter percentage more nearly agrees with our observations on the subject.

Sex incidence

As regards sex incidence, of 42 cases, 8 only were females. I do not think that this preponderance of male cases is in any way due to the objection of the natives to bring their women folk for treatment, as seclusion of the female sex is not carried to such an extent in these districts as in Egypt. Many women who were unable to come for treatment or whose husbands were unwilling for them to leave their houses were visited in their houses.

Race incidence

As far as can be seen no special race shows a predisposition for the disease, all seem to be equally prone to infection.

The following table shows the race incidence :—

	Positive Kala-azar	Microscopically negative, clinically positive	Possible cases by native report
Arab	9	4	8
Sudanese	7	3	6
Egyptian	2	Nil	Nil
Abyssinian	1	1	Nil
Berbereen	1	Nil	Nil
Total	20	8	14

Seasonal incidence

As cases have been seen at various seasons of the year, it is difficult to determine the actual time of infection. From information gathered from native sources the disease would seem to show itself at the end of the rains; this would seem to favour infection taking place during the rains, the cool season.

It must be remembered that during December and January the temperature is generally low, and, assuming that a low temperature is a necessary factor in infection, it is quite possible that the disease may be contracted during these months and, lying dormant through the hot weather, only asserts itself during the following rains.

This would point to a prolonged quiescent period, though, experimentally, it has been found possible to demonstrate Leishman-Donovan bodies by spleen puncture within fifty days of infection.

As regards family distribution the disease would appear to differ from that seen in India.

In only one case was there any history of infection of the second member of a family.

Is Kala-azar curable?

The question as to whether the disease is curable is of the utmost importance and, whether a permanent cure is possible or not, is a moot point.

The following four cases which we have seen, are of interest, as they were reported as positive last year by Captain Bousfield.

Saleh Mohammed has been seen three times since last November, and on each occasion has been fit and well. On the first occasion on which he was seen both spleen and liver puncture proved negative.

The patient suggested to us that he should come again next day for further punctures as he said that since he had been first punctured, six months before, he had had no sign of fever, and this he attributed to the puncture.

Shawish Tisama was seen in February, 1910, about ten months since he was diagnosed kala-azar. He was fit and well. Spleen puncture was negative for Leishman-Donovan bodies.

Ramadan Someet, a boy about ten, seen at Gallabat a year after original diagnosis was made. He was fit and well with no fever, and spleen barely palpable.

Ali Abdel Kader. This case was diagnosed as suspicious by Captain Bousfield. When seen by us he had fever, enlarged spleen, and leucopenia. Leishman-Donovan bodies were present in the peripheral blood. He was treated with tincture of senega and, after three months' treatment, fever had ceased and parasites had disappeared from the peripheral blood. After six months' treatment the spleen was not palpable.

The treatment which these cases had was as follows:—

The first had a short course of quinine, apparently about 25 grains a day, for five days.

The second was treated first with regular doses of quinine and since November has been taking tincture of senega regularly.

The third was treated with quinine for a period of some months.

The fourth was treated entirely with tincture of senega for 5 months. None of these cases, as far as I could find out, had had any suppurative focus, or anything wrong with them which would tend to create a leucocytosis, since they were diagnosed kala-azar.

Are these four cases of spontaneous cure of the disease; is the disease simply dormant in their systems in some form of which we know nothing, or is spleen puncture of therapeutic value in some instances?

I have frequently heard natives say that spleen puncture, even in cases of malarial splenomegaly, has improved their condition and reduced the size of the spleen.

A focus of bilharziosis

A point of considerable interest, which was noted during the tour west of Singa, was the enormous number of cases of bilharziosis.

All the villages lying on Khor Um Esh would appear to be more or less infected.

This khor, which has a shallow, ill-defined bed, runs in a north-easterly direction for about 20 miles and joins the Blue Nile at Abu Shoka. During the rains it is always full and is fed by many subsidiary khors, of a similar nature to the main khor, which drain a large area. During the dry season the people dig wells in the bed of the khor, getting water about 30 feet from the surface.

In the greater percentage of cases seen, children, chiefly boys, from 5 to 15 years of age, were infected, though both men and women also suffer from the disease.

Patients complain of "Bouul Har" or scalding urine. Microscopic examination of the urine showed numbers of bilharzia ova.

The children all complained of frequent micturition and irritation of the bladder; some cases I saw passed large clots of pure blood and a very small quantity of urine with them.

Others undoubtedly, from their symptoms, had infection of the rectum as well, and were passing blood and mucus.

Several women whom I saw suffering from this disease also complained of renal and ovarian pains, which may have meant an infection of these organs also.

The heavy infection of this definite area with bilharzia is of exceeding interest as, up to the present, only isolated cases of bilharziosis in Sudanese and Arabs have been recorded, and so far as I know no definite infected area has been reported. Samples of water were taken from the wells of Hillet Howeires, in which at least two-thirds of the boys appear to be infected.

The examination of the water showed considerable deposit, chiefly earth and sand, with some vegetable debris. Flagellated and ciliated bodies were present in considerable numbers.*

Recommendations

Recommendations

1. That a special-service British Medical Officer with a knowledge of kala-azar be appointed to Sennar Province for the further investigation of this disease. That he be given full power of isolating cases and contacts as he may think fit.

2. That he make further experiments to determine, if possible, the mode of infection. We think that, owing to the wide-spread distribution of the cases and the absence of epidemics, animal experiments, which we have found to be easy and practicable, promise the best results.

3. All Medical Officers serving in infected districts should be thoroughly cognisant of the clinical features of the disease and instructed in making proper peripheral blood films, which they should send to some competent authority for examination.

4. In infected areas all sanitary barbers who have not been as yet instructed in the symptoms of the disease, should be instructed therein without delay.

5. They should be ordered to report all suspicious cases to the Medical Officer of their district, and, as an incentive to the discovery of all cases, they should be given a reward of P.T. 25 for every positive case reported.

6. Officials and employees of the Government suffering from fever should not neglect such attacks, especially if persistent, but consult a Medical Officer. It is possible that kala-azar in its early stages is a curable disease.

7. All officers, employees, etc., should take prophylactic doses of quinine during the *Kharif*.

8. Owing to the, as yet, uncertain method of transmission of the disease, preventive measures of a general sanitary nature should be generally carried out.

(*a*) Personal cleanliness and house cleanliness are of importance. Should the disease be carried from person to person neglect of this fact is dangerous.

(*b*) Especial attention should be given to absolute cleanliness of kitchens, cooking and table utensils and food. Water should be filtered or boiled.

(*c*) Cleanliness of villages is essential, and we would suggest that Sheikhs be held responsible for this. A dirty and insanitary village might be the starting point of an epidemic.

* Enquiry showed that infection in all probability was acquired through the skin owing to the habit of paddling about in the infected pools formed round the wells.—A.B.

(*d*) The present system of putting animals at night in zerebas adjoining the owner's *tukl*, and in some cases in their *tukl*, is to be condemned.

(*e*) That *tukls* of infected cases and all such belongings as cannot be thoroughly disinfected be burnt.

(*f*) Should an extensive outbreak of kala-azar occur in any village, the whole village should be burnt and a new village built on a fresh site, after the patients and contacts have been isolated.

9. A careful record of all cases of kala-azar should be sent half-yearly by Senior Medical Officers of Provinces to the Principal Medical Officer.

Conclusion

In conclusion, we wish to express our best thanks to the Principal of the Gordon College and the Director, Wellcome Tropical Research Laboratories, for so kindly placing their steamer and floating laboratory at our disposal.

To the Governor and Officials, both English and Egyptian, of Sennar Province for the great assistance they have given us in carrying out our investigations. To Captain Bousfield, R.A.M.C., whose report on kala-azar was most useful to us in our researches, and to El Yousbashi Nesib Eff. Barudi, S.M.D. for his invaluable help both in our clinical and pathological work in Singa.

CASES OF KALA-AZAR FOUND BY COMMISSION

Name	Sex	Age	Nationality	Village	Markaz	Province	Peripheral Blood	Spleen Puncture
Ali Wad Toto	M.	10	Sudan.	Wad El Aies	Singa	Sennar	+	+
Gasm El Seed Wad Hay Doub ...	M.	10	Arab	Abu Hogar	Singa	Sennar	+	+
Fatma Bint Izz	F.	13	Arab	Barankwa	Abu Naama	Sennar	+	+
Abdl Sadik Wad Omar	M.	14	Arab	Barankwa	Abu Naama	Sennar	+	+
3027 Nafar Mohd., Abdel Rahim, Wks. Dept.	M.	23	Egyp.	Gedaref	Gedaref	Kassala	—	+
1225 Nafar Osman Bergaz, A. Bn.	M.	25	Arab	Gedaref	Gedaref	Kassala		+
Ali Eff. Gadein Katib	M.	25	Sudan.	Gallabat	Gallabat	Kassala	+	+
Abdulla Wad Barood	M.	10	Sudan.	Taufikia	Singa	Sennar	+	+
Sabah El Kheir Wad Tiah ...	M.	12	Sudan.	Mansoura	Singa	Sennar	+	+
Zeinab Bint Bakhat	F.	11	Arab	Hariri	Singa	Sennar	+	+
Ahmed Adam Adam	M.	33	Arab	Karkoj	Abu Naama	Sennar	+	+
Ibrahim Wad Beshir	M.	11	Arab	Launi	Abu Naama	Sennar	+	
Egyptian	M.	34	Egyp.	Abu Naama	Abu Naama	Sennar	+	+
Gasmallah Mohd.	M.	19	Arab	Mafaza	Mafaza	Kassala	+	+
Kassala Abu Gaada...	M.	25	Sudan.	Roseires	Roseires	Sennar		+
Mohd. Ewad El Seed	M.	12	Arab	Shellal	Singa	Sennar	—	+
Yusef El Tigani	M.	9	Arab	Tangaru	Abu Naama	Sennar	+	+
Yadisa Kataba	M.	19	Abys.	Singa	Singa	Sennar	+	+
Abbas Abdel Beyen...	M.	10	Arab	Singa	Singa	Sennar	+	+

CASES OF KALA-AZAR FOUND BY COMMISSION—*continued*

Date seen	Duration of Illness	Result	Complications	Remarks
Nov. 25, 1909	1 month	Died, May 16, 1910	Wasting. Slight œdema of feet and face	
Dec. 11, 1909	1 month	Died, Jan. 5, 1910	Œdema of feet and face. Wasting slight	
Nov. 19, 1909	5 months	Died, Jan. 9, 1910	Œdema of feet and face. Wasting marked. Diarrhœa	
Nov. 19, 1909	14 days	Died, Jan. 26, 1910		Had intermittent attacks of Fever for 2 months
Feb. 19, 1910	6 weeks	Died, April 1910	Cancrum Oris. Œdema of feet and face. Wasting	
Jan. 13, 1910	1 month	Died, Jan. 28, 1910	Cancrum Oris. Diarrhœa. Œdema of feet and face	
Jan. 26, 1910	6 weeks	Died, April 2, 1910		
Dec. 9, 1909	6 weeks	Died, Dec. 17, 1909		
Nov. 26, 1909	3 months	Died, Dec. 10, 1909	Œdema of feet and face. Wasting	
Dec. 6, 1909	3 months	Died, Dec. 19, 1909	Pigmentations marked	
Dec. 17, 1909	15 days	Died, Dec. 3, 1909		
Dec. 12, 1909	2 months	Died, Dec. 14, 1909	Œdema of feet and face. Wasting. Apathetic	Case too ill for spleen puncture, spleen was barely palpable
1909	3 months	Died, 1909		Transferred to Cairo
Feb. 15, 1910	1 month	Died, Mar. 20, 1910		
—	2 months	Died, Oct. 11, 1909	Diarrhœa. Œdema of feet and face	This case was treated in Roseires and diagnosed on a post mortem spleen smear
May 27, 1910	5 months	Died, June 23, 1910	Œdema of feet and face. Wasting	
July 26, 1910	4 months	Died, July 26, 1910	Cough with bloody expectoration	Practically *in extremis* when seen
July 26, 1910	2 months	Died, July 31, 1910	Perforating ulcer, large intestine	Sudden perforation of an ulcer at splenic flexure
April 19, 1910	9 months	Died, June 20, 1910		

CASES OF KALA-AZAR CLINICALLY POSITIVE

Name	Sex	Age	Nationality	Village	Markaz	Province	Result	Remarks
Nura Bint Seyid ...	F.	7	Sudan.	Berta	Abu Naama	Sennar	Died	Patient too ill to remove or to have spleen puncture performed. Peripheral blood negative
Mohammed Wad Omar	M.	5	Arab	Adam Hourain	Singa	Sennar	Died	Ditto
Ali Wad Omar	M.	7	Arab	Launi	Abu Naama	Sennar	Unknown	Clinically typical. Peripheral blood negative. Improved under quinine
Abdelnabi Gebara ...	M.	9	Arab	Lueisa	Abu Hashim	Sennar	Unknown	Clinically typical. Peripheral blood negative. Too ill to move or to have spleen puncture performed
Abdel Fattah Saîd ...	M.	26	Sudan.	Roseires	Roseires	Sennar	Died	A post mortem spleen smear was made by M.O., Roseires, but lost. Case stated to be identical with other case which died in hospital and was positive
Khadmallah Bint Rehan	F.	10	Sudan.	—	—	Sennar	Died	Reported by M.O., Roseires
Abdel Hamid El Wahed	M.	50	Arab	Keneiza	Abu Naama	Sennar	Died	Enormous ascites with pressure œdema. Tapped twice. Improved greatly. Died about a month afterwards

CASES REPORTED AS KALA-AZAR BY NATIVES

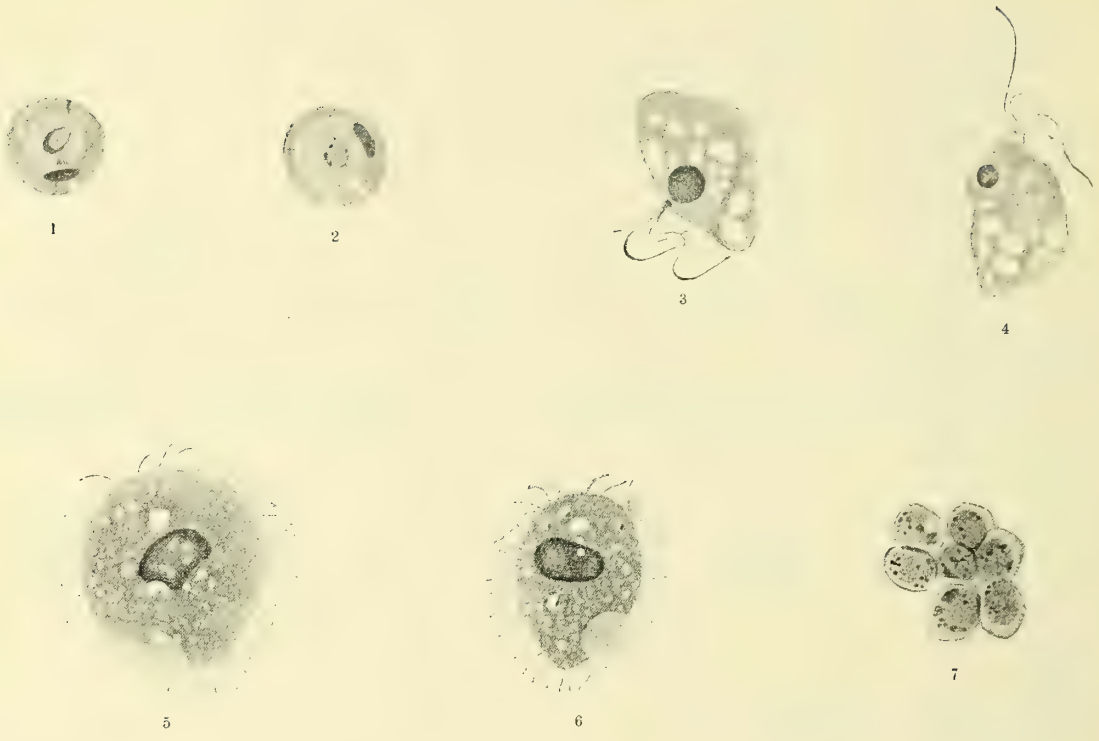
Name	Sex	Age	Nationality	Village	Markaz	Province	Duration of Illness and Result
Fatima Bint Seyed ...	F.	13	Sudan.	Attib	Abu Naama	Sennar	Died, 5 months
Tindel Wad Dafter ...	M.	11	Sudan.	Wad Daftar	Gedaref ...	Kassala	Died, 3 months
Ahmed Mohd. Ali Atalla	M.	30	Arab	Kakoum	Abu Hashim	Sennar	Died, 2 years
Suleiman Mohd.	M.	40	Tama Arab	Tama	Abu Naama	Sennar	Died, 3 months
Baklat Ali	M.	35	Tama Arab	Tama	Abu Naama	Sennar	Died, 3 months
Hageiza Bint Eturia ...	F.	30	Sudan.	Tama	Abu Naama	Sennar	Died, 2½ months
Ataminno	F.	30	Sudan.	Tama	Abu Naama	Sennar	Died, 2 months
Abdel Rahim Wad Farag	M.	1	Sudan.	Tama	Abu Naama	Sennar	Died, 1 month (son of previous case)
Amina Mamallah	M.	28	Sudan.	—	Abu Hashim	Sennar	Died, 3 months
— Bint Hammam ...	F.	8	Arab	Bados	Roseires	Sennar	Died, 1½ months
Mohd. El Teyeb	M.	50	Arab	Lueisa	Abu Hashim	Sennar	Died, 5 months

Also:—Two Cases, names not obtainable, from Basiak Arabs.
One Case, name not obtainable, from Sudanese.

RESULT OF CASES OF KALA-AZAR REPORTED BY CAPTAIN BOUSFIELD, R.A.M.C.

Name	Sex	Age	Nationality	Village	Markaz	Province	Positive	Suspicious	Present Condition	Remarks
Saleh Mohammed	M.	23	Arab	Abdin	Singa	Sennar	+	—	Healthy	Seen Nov. 15, 1909 and May 29, 1910. Says he has not been ill a day. Seen twice by Captain Bousfield in April, 1909
Ismail Mohammed	M.	...	Arab	Abdin	Singa	Sennar	—	+	Healthy	Spleen puncture showed no Leishman-Donovan bodies
612 Shawish Tisama	M.	30	Arab	...	Gedaref	Kassala	+	—	Healthy	
Ramadan Someet	M.	11	Sudan.	Gallabat	Gallabat	Kassala	+	—	Healthy	
687 W.O. Goma Sadik	M.	30	Arab	...	Gedaref	Kassala	—	+	Healthy	Spleen puncture showed no Leishman-Donovan bodies
Hamed Wad Hamed	M.	...	Arab	Abu Gabid	Gedaref	Kassala	+	—	Dead	
Hawwa Bint Elias	F.	...	Arab	Sherif Seyed	...	...	—	+	Dead	
270 W. O. Mohd. Mahmud	M.	...	Arab	...	Gallabat	...	—	+	Healthy	
Dustabarra	M.	...	Abyss.	...	...	...	—	+	Healthy	
Saleh Ibrahim	M.	...	Arab	...	...	...	—	+	Dead	
Beshir Adam	M.	...	Abyss.	...	...	...	—	+	Healthy	
Wife and child of above	F.	...	Abyss.	...	...	...	—	+	Healthy	
Mohammed Ahmed	M.	...	Arab	Doka	Gedaref	...	—	+	Dead	
Mustafa Mohammed	M.	...	Sudan.	Galet El Nahl	...	...	+	—	Dead	
Maghbone El Mohd	M.	...	Arab	Mafaza	Mafaza	...	—	+	Dead	
Mohd. Ali Nower	M.	...	Arab	Geili	...	...	—	+	Dead	
160 Mohd. Idris KPP...	M.	...	Arab	Mafaza	...	...	—	+	Healthy	
Mohd. Wad Gadulla	M.	...	Arab	Lueisa	Abu Hashim	Sennar	+	—	Dead	
Kazab Abdulla	M.	...	Arab	Singa	Singa	...	+	—	Dead	
1393 Onb. Mahfouz Ayoub	M.	...	Egyp.	...	...	...	+	—	?	Transferred to Cairo
Abdulla Latif	M.	...	Arab	Singa	...	...	+	—	Healthy	
Abdel Kheir	M.	...	Sudan.	Abdin	...	...	+	—	Dead	
Jak Seyid Hegazi	M.	...	Arab	Sennar	Sennar	...	—	+	Healthy	
Babiker El Hag	M.	...	Arab	...	...	...	+	—	Dead	

PLATE X



M. Rhodes

1—4, ×1000 *diam.*; 5—6, ×800 *diam.*; 7, ×1500 *diam.*; 8—12, ×2000 *diam.*

Protozoa other than Kala-azar met with by the Commission

1—2. Cyst-like bodies from fæces, case of kala-azar. Probably one stage in figs. 3 and 4.

3—4. Trichomastix bodies from same case.

5—6. Balantidium found in two cases, from spleen puncture and from the mucous membrane of the large intestine. Adult parasite.

7. Young forms of 5 and 6.

8—9. Flagellated bodies in distilled water.

10—12. Flagellated bodies from fæces of monkey infected with kala-azar.

Kala-azar Commission

to investigate the Prevalence and Cause of the Disease in the Eastern Sudan

(2) Pathological Report

by

Lieutenant W. E. Marshall, M.B., Ch. B., D.P.H., R.A.M.C.

Attached Egyptian Army

Clinical Notes

The material for carrying out the pathological investigation into kala-azar in Sennar Province was obtained for the most part from the 12 cases of kala-azar admitted into Singa Hospital between the months of December, 1909, and July, 1910.

The clinical histories of these cases were briefly as follows:—

Clinical notes

Case 1. A.A.B., boy, aged 10. Admitted into hospital on April 19, 1910, with enlargement of the spleen, irregular fever, and an œdematous swelling of the right cheek. His condition remained the same till May 21, 1910, when the fever became more irregular, jaundice set in, and he died suddenly on June 20, 1910. He had a progressive leucopenia, the white blood corpuscles numbering 5200 on April 24, 1910; 4531 on May 3, 1910; 4375 on May 14, 1910; and 2200 on May 26, 1910. Leishman-Donovan parasites were found in the peripheral blood and by spleen puncture. Post mortem examination showed enormous enlargement of the spleen with white patches on the capsule and some adhesions at the upper end. The liver was also enlarged. The bone-marrow was red but the intestines appeared healthy. Treated with Tinct. Senega.

Case 2. A.S.W.O., boy, aged 14. Admitted into hospital on Dec. 7, 1909, with enlargement of the spleen, fever and emaciation. He had had occasional rigors before admission. He became deaf without associated pain, and suffered from night terrors. His temperature was very high and very irregular, and he died on Jan. 26, 1910. The leucocytes numbered 4500. Leishman-Donovan parasites were found in the peripheral blood and by spleen puncture. Treated with tincture of senega and quinine.

Case 3. A.W.T., boy, aged 10. Admitted into hospital on Nov. 30, 1909, with splenomegaly, constant fever, and œdema of the feet. He did fairly well, and had periods of apyrexia, the temperature sometimes remaining normal for four clear days. He went home on April 23, 1910, but died there on May 16, 1910. He had albuminuria and also suffered from tapeworm, the ova of *Tænia nana* and *Tænia solium* being both present in the fæces. Leishman-Donovan bodies were found in the peripheral blood and by spleen puncture. Treated with tincture of senega and quinine.

Case 4. F.B.A., girl, aged 13. Admitted into hospital on Dec. 7, 1909, suffering from diarrhœa, splenomegaly, fever and emaciation. She had attacks of epistaxis, and suffered from suppuration in the middle ear. She died suddenly on January 9, 1910. Leishman-Donovan bodies were found in the peripheral blood and by spleen puncture. Treated with tincture of senega.

Case 5. S.K.W.T., boy, aged 12. Admitted into hospital on Dec. 1, 1909, with fever, swelling of face and feet, and splenic pain but no splenic enlargement. History of rigors every second day at 4 in the afternoon. Leucocytes 5859. He died suddenly on Dec. 10, 1909, complaining of severe pain in the abdomen and chest. Leishman-Donovan bodies were found by spleen puncture, and in the peripheral blood. Treated with tincture of senega and quinine.

Case 6. A.A.K., boy, age about 12. Admitted into hospital on Dec. 1, 1909, with fever, splenomegaly, slight enlargement of the liver, and leucopenia, the white blood cells numbering 3125. After a short stay in hospital he was allowed to attend as an

Clinical notes

out-patient and steadily improved. On Feb. 27, 1910, he was able to return to school and on April 25, 1910, the splenic enlargement had disappeared. Leishman-Donovan bodies were found in the peripheral blood. Treated with tincture of senega.

Case 7. Z.B.B., girl, aged 11. Admitted into hospital on Dec. 7, 1909, with enormous enlargement of the spleen, fever, extreme emaciation and pigmentation of the skin. Liver slightly enlarged. Died on Dec. 19, 1909. Leishman-Donovan parasites found in the peripheral blood. Treated with tincture of senega.

Case 8. A.W.B., boy, aged 10. Admitted into hospital on Dec. 11, 1909, suffering from fever, enormous enlargement of the spleen, and œdema of the face. Died on Dec. 17, 1909, six days after admission. Leishman-Donovan parasites found in the peripheral blood and in the spleen. Treated with tincture of senega.

Case 9. G.E.S.M., boy, aged 11. Admitted into hospital on Dec. 13, 1909, with enormous splenomegaly, fever, œdema of the feet and face, and slight emaciation. Died on Jan. 5, 1910. Leishman-Donovan parasites found in the peripheral blood and in the spleen. Treated with tincture of senega.

Case 10. Y.E.T., boy, aged 10. Admitted into hospital on July 26, 1910, with splenomegaly, fever, œdema of face and feet, and cough with blood-stained expectoration. He died on the day of admission. Leishman-Donovan parasites found in the peripheral blood and by spleen puncture.

Case 11. Y.K., boy, aged 19. Admitted into hospital on July 25, 1910, with splenomegaly and slight fever. He also complained of splenic pain. Two days after admission he complained of severe pain in the abdomen, and his temperature rose. The pain continued, diarrhœa set in and he died on July 31, 1910. Leishman-Donovan parasites found in the peripheral blood and in the spleen. Treated with tincture of senega. Post mortem examination showed acute general peritonitis from rupture of an ulcer in the upper part of the descending colon.

Case 12. M.E., boy, age about 12. Admitted into hospital on May 31, 1910, with high fever, splenomegaly, œdema of the feet and face, and slight emaciation. He died on June 23, 1910. Leishman-Donovan bodies found by splenic puncture but not in the peripheral blood. Treated with tincture of senega.

Pathological material was also sent by Captain Thomson from cases seen by him which were not admitted into Singa Hospital. The outstanding clinical features of the disease in this Province are splenomegaly, fever, and œdema of the face and feet. With the exception of Case 7, the emaciation was usually slight, this being probably accounted for by the comparatively short duration of the illness. Dyspnœa was a common terminal symptom. Diarrhœa was present in two cases, epistaxis in one, and one died from rupture of an ulcer in the descending colon. One case suffered from extreme deafness and another from middle ear disease. (We have since seen in Khartoum Military Hospital a boy with kala-azar who also had suppuration in the middle ear.) Splenic pain was frequently complained of and death was often sudden. Two cases had rigors. The fever was usually irregular, the maximum rise occurring about 4 in the afternoon.

Treatment. In view of Major Ensor's favourable result with Tinct. Senega this drug was tried in the treatment of the disease. It was given in doses of $\frac{1}{2}$ to 1 drachm 3 times a day. When rigors were or had been present, quinine was also given. One of the cases, No. 6, has apparently recovered, and senega was the only drug used. In another case (Case 3) there appeared to be a marked diminution in the number of parasites found by spleen puncture, and in the same case the flagellated bodies found in the fæces, and to be described later, disappeared after its administration and reappeared again when it was stopped, so it would seem to have a definite toxic effect on protozoa.

Peripheral Blood

Presence of Leishman-Donovan bodies in the peripheral blood

(*a*) *Presence of Leishman-Donovan bodies in the peripheral blood*

Out of 15 cases of kala-azar in which the peripheral blood was examined the parasites were present in 13 or in 86·6 per cent. In one of the negative cases only 4 slides were

available for examination. Out of these 13 cases, in 9 the parasites were present in the large mononuclear cells only, in 3 they were found only in the polynuclear cells, and in one case in both. They were never found in very large numbers seldom averaging one parasite per slide. We have found as many as 4 inside one large mononuclear cell. There seems to be a definite relation between the number of parasites in the blood and the number in the spleen. In the negative cases the parasites were in small numbers in the spleen and in Case 2, where the parasites were also scanty in the spleen, they were not found in the peripheral blood until the eighth film had been examined. In the majority of positive cases, however, the examination of 3 slides is usually sufficient for diagnostic purposes. The parasites did not appear to vary with the time of day nor with the fever, nor could we find them markedly increased before death.

(*b*) *Increase in the large mononuclear leucocytes*

Increase in the large mononuclear leucocytes

This as a rule was well marked, varying from 8·19 per cent. to 38·21 per cent. of the white cells. The case with the lowest percentage of these cells was the one which recovered. There was frequently marked vacuolation of these mononuclear cells and some were comparatively of an enormous size, one measuring 34μ by 39μ being found on one occasion.

(*c*) *Other blood changes*

Except in Case 6 there was a definite diminution in the number of polynuclear cells. Large deeply basophile mononuclear cells were frequently present. These, in our opinion, are plasma cells. The nucleus is excentrically placed, there are small vacuoles in the protoplasm, and the perinuclear zone, so characteristic of these cells, is well marked. The wheel-shaped structure of the nucleus was absent, but this does not occur, according to Naegeli, when these cells are present in the peripheral blood. It is difficult, however, in the peripheral blood to distinguish the above from Naegeli's myeloblasts. Nucleated red cells were occasionally found and megalocytes were common. Polychromatophilia was often well marked.

(*d*) *Relation of eosinophilia to kala-azar*

Relation of eosinophilia to kala-azar

In well-marked cases of kala-azar there is undoubtedly a diminution in the number of eosinophile cells present in the peripheral blood. It is interesting to note, however, that a great many cases of fever and splenomegaly suffered from eosinophilia. Out of 43 such cases where the peripheral blood was examined 18 showed eosinophilia, 2 showed eosinophilia and quartan malaria parasites, 2 showed quartan malaria only, and 21 showed no special change. Another interesting fact is this:—2 cases of kala-azar diagnosed positive by Captain Bousfield, and 2 cases put down by him as suspicious (one, No. 6 of this series) were seen by us. The first two were both healthy. One (S.M.) had an eosinophilia of 8·82 per cent., and in the other (R.S.) the eosinophilia is noted as "enormous." Of the two suspicious cases one (G.S.) showed definite eosinophilia in the peripheral blood; the other (No. 6 of this series) had, when first examined, an eosinophilia of 2·86 per cent., but after recovery he also showed an increase in the eosinophile cells. The brother of Case 2, who had splenomegaly, also showed eosinophilia in the peripheral blood and a local eosinophilia in the spleen. The sister of Case 6, suffering from fever and splenic enlargement, had also eosinophilia in the peripheral blood. Two Abyssinians were admitted into Singa Hospital suspected to have kala-azar. One, Case, No. 11, was proved positive; the other, whose symptoms suggested the disease, has

so far shown no parasites in the spleen or peripheral blood and is still under observation in Singa Hospital. Eight more Abyssinians lived with the above and all were examined. One had an enormous spleen but the others appeared healthy. The peripheral blood was examined in all cases with the following results:—

No. 1	11·1 per cent. eosinophiles
„ 2	10 „ „
„ 3	30 „ „
„ 4	3·2 „ „
„ 5	2·7 „ „
„ 6	8 „ „
„ 7	33·3 „ „
„ 8	13·7 „ „

Urine and Fæces in Kala-azar

Examination of these was carried out in order to determine, if possible, if the kala-azar parasite leaves the body with the excreta. In the urine, albumin was twice present, but microscopical examination failed to reveal anything abnormal. In the fæces no kala-azar parasites were found. In 4 of the 8 cases examined, however, flagellated bodies were present in the fæces, in 3 of them in enormous numbers. In hanging-drop preparations these are seen as actively motile bodies darting backwards and forwards across the field. As they gradually lose their motility they become actively amœboid and throw out long narrow pseudopodia. In specimens stained by Leishman's method they appear as oval bodies varying greatly in size but averaging about 13μ by 11μ. They appear to have 4 flagella (sometimes only 3 can be seen) coming off near the nucleus, and one flagellum is longer than the others (Plate X., figs. 1–4). In one of the small forms, measuring 5·9μ by 10·2μ, the longest flagellum measured 13·6μ in length, and in another similar parasite the flagellum measured 20μ in length. The protoplasm is vacuolated and there appear to be bacteria, etc., in the interior of the parasite. They remain alive and actively motile at 22° C. for at least 6 days. They were best found in the fluid portion of the stool after an aperient had been administered. If not originally present, cultivation at 22° C. did not show any parasites.

Flagellated bodies in fæces

In Case 3, the ova of *Tænia nana* were constantly present in the stools and the ova of *Tænia solium* were also found on two different occasions. Actively motile spirochætes were also frequently found.

In order to determine if these flagellated bodies are common in the alimentary canal of the inhabitants, numerous controls, numbering 35, were examined. Twenty of these were school-boys attending school, 3 were apparently healthy young children, and the remainder were patients sick with fever and other illnesses. Flagellated bodies resembling the above were found in 5 cases but always in small numbers. They appeared similar to the above but they were present in such small numbers that microscopical examination of stained specimens was impossible. Three of the positive cases were school-boys; No. 1, Spleen normal, no fever, quartan malaria and eosinophilia in his blood; No. 2, Spleen normal, no parasites in peripheral blood; No. 3, Spleen enlarged, no fever, quartan malaria and eosinophilia in the peripheral blood. Of the other two, one was a woman admitted

Somewhat similar bodies found in healthy controls

into hospital with quartan malaria and slight splenomegaly, while the other was a child, an out-patient with diarrhœa. Out of these 35 cases the ova of *Tænia nana* were found in 4 cases—all school-boys—and the ova of *Fasciola ægyptica* in one case of fever and splenomegaly.

The presence of four free flagella and the absence of an undulating membrane would indicate that these bodies are trichomastix forms of trichomonas. The flagella usually arise from the blunt end of the parasite, but sometimes from the side. The vacuolation of the protoplasm, so well marked in the larger forms, is usually indistinct or absent in the smaller forms of the parasite. Bodies resembling the so-called cysts of trichomonas were also present along with these trichomastix bodies and are shown in Plate X., figs. 1–2.

Previous notes of flagellates in fæces

Flagellated bodies have been frequently found in the human alimentary canal, and are generally supposed to be harmless inhabitants of that canal. Castellani thought that, though their presence in small numbers might be harmless, their presence in large numbers was the ætiological factor in two cases of diarrhœa in Ceylon. Still more recently Castellani and Chalmers have found another flagellate in the fæces in cases of diarrhœa. It has two flagella and an undulating membrane and has been called by them *Bodo asiaticus*. Wenyon has recently found a new flagellate, *Macrostoma mesnili*, in the fæces of a native of the Bahamas. It has three flagella and a large cytostome. It appeared to have no ill-effects upon the host.

Cummins noted the presence of flagellated bodies in the fæces of a case of kala-azar occurring in the Sudan. He also stated that he had seen similar bodies in a case of acute dysentery.

A young dog was fed frequently with milk mixed with the fæces of kala-azar patients, sometimes with and sometimes without flagellated bodies. It remained perfectly well and was chloroformed 54 days after the first feeding. There was no evidence of infection with kala-azar.

The presence of Leishman-Donovan Bodies in Dogs and other animals

In view of Nicolle's discovery of spontaneous Leishmaniosis in the dog, recently confirmed by Gabbi and Basile in Italy, Critien in Malta, Alvarez in Lisbon, and the Sergents in Algiers, 21 dogs from the village of Singa were killed and examined. Nine of these were emaciated but showed no symptoms of illness, 2 were emaciated and ill, and 10 were normal. One of the latter was from a *tukl* containing a case of kala-azar. In 9 cases smears were made from the spleen, liver and heart's blood, in 11 cases smears were made from the spleen and bone-marrow, and in one case from the spleen and liver. All were negative, no kala-azar parasites being found. We recognise that the number of dogs examined was too small to say definitely that spontaneous Leishmaniosis does not occur here in the dog. In the dog living in the infected *tukl* the spleen was enlarged, but careful search failed to reveal the presence of Leishman-Donovan bodies. Spleen smears were also examined from the spleens of 4 healthy cows, 3 healthy goats and 15 healthy sheep, all with negative results.

Kala-azar a natural infection in dogs

There is now no doubt that Leishmaniosis occurs in dogs as a natural infection, and Nicolle's discovery has been confirmed in different parts where infantile kala-azar is found. Basile recognises two different forms of natural infection in the dog, the acute and the chronic, and he considers that the former, which affects young dogs and lasts from 3 to 4 months, plays the more important part in the spread of the disease.

TABLE I

Animal	Where inoculated and date of inoculation	Material inoculated	Course of Disease	Result	Organs infected	Remarks
Monkey B	Liver and peritoneum Jan. 9, 1910	Post mortem spleen puncture Case 4	30th day: Liver puncture negative 53rd day: Liver puncture negative 62nd day: Chloroformed	Infected	Spleen + Liver —	Spleen slightly enlarged. Parasites present in moderate numbers
Monkey C	Peritoneum March 12, 1910	Post mortem spleen emulsion Monkey B	8th day: Peripheral blood negative 22nd day: Peripheral blood negative 33rd day: Peripheral blood negative 45th day: Peripheral blood negative Liver puncture negative 66th day: Liver puncture negative 94th & 95th days: Liver puncture negative 108th day: Chloroformed	Not infected	Spleen — Liver — Bone-Marrow —	
Monkey D	Peritoneum April 21, 1910	Spleen puncture Case 3	14th day: Peripheral blood negative 55th day: Liver puncture negative 63rd day: Ill, dyspnœa, splenomegaly Liver puncture negative Spleen puncture negative 65th day: Died	Infected	Spleen + Liver + Bone-Marrow +	Miliary tuberculosis also present. Spleen markedly infected with tubercle
Monkey E	Peritoneum April 23, 1910	Spleen puncture Case 1	14th day: Peripheral blood negative 54th day: Liver puncture positive 58th day: Peripheral blood negative 78th day: Spleen enlarged Spleen puncture positive Liver puncture negative Peripheral blood negative 143rd day: Diarrhœa Liver puncture positive 145th day: Chloroformed	Infected	Spleen + Liver + Bone-Marrow +	Monkey emaciated. Spleen enlarged. Liver enlarged and cirrhotic Bone-Marrow red. Intestinal intussusception present
Monkey H	Peritoneum June 21, 1910	Post mortem spleen emulsion Case 1	65th day: Chloroformed	Not infected	Spleen — Liver — Bone-Marrow —	The spleen emulsion from Case 1 was made 8 hours after death

TABLE I—*continued*

Animal	Where inoculated and date of inoculation	Material inoculated	Course of Disease			Result	Organs infected	Remarks
Monkey K	Subcutaneous tissues July 11, 1910	Spleen puncture Monkey E	160th day Spleen enlarged Liver puncture negative	161st day Spleen puncture negative	163rd day Spleen puncture negative			Still alive
Monkey L	Intravenous and subcutaneous July 11, 1910	Spleen puncture Monkey E		161st day Spleen enlarged Spleen puncture positive		Infected		Still alive
Monkey N	Peritoneum July 11, 1910	Spleen puncture Monkey E	120th day Liver puncture doubtful		155th day Died	Infected	Spleen + Liver + Bone-Marrow +	Small ulcer in small intestine. Smears and sections of this negative
Monkey O	Peritoneum July 26, 1910	Post mortem spleen puncture Case 10			42nd day Found dead	Not infected	Spleen — Liver — Bone-Marrow —	Spleen not enlarged
Monkey P	Peritoneum July 31, 1910	Post mortem spleen emulsion Case 11	123rd day Liver puncture positive		134th day Died	Infected	Spleen + Liver + Bone-Marrow +	
Monkey R	Subcutaneous tissues July 31, 1910	Post mortem spleen emulsion Case 11	150th day Spleen enlarged Liver puncture positive		156th day Peripheral blood positive	Infected		Still alive
Monkey S	Not inoculated	Kept in cage beside Monkey N	112th day Ill	117th day Liver puncture positive	121st day Died	Infected	Spleen + Liver + Bone-Marrow +	Lived continuously with Monkey N, became naturally infected and died before Monkey N

Experimental Kala-azar in the Grey Monkey (*Cercopithecus sabæus*)

Inoculation experiments were carried out to determine the infectivity of the ordinary grey monkey of the Sudan (*Cercopithecus sabæus*) to kala-azar, and the results are shown in Table I. (*pages* 162–163).

Twelve monkeys were used in the experiments. Eight were infected with kala-azar. In 3 the experiment failed, and one still remains doubtful. So far we have found that the best method of infecting these monkeys is from a splenic puncture during life. An ordinary sterilised hypodermic syringe is taken, washed out with sterile citrate, spleen puncture carried out in the ordinary way and the contents of the syringe immediately injected into the peritoneal cavity of a monkey.

Infected cases

Infected cases

Of the 8 infected cases, 5 were infected intraperitoneally, one subcutaneously, one subcutaneously and intravenously, and one was naturally infected.

Of those infected intraperitoneally, Monkey B was chloroformed on the 62nd day. Post mortem examination showed enlargement of the spleen and Leishman-Donovan bodies present in moderate numbers. No parasites could be found in the liver, so probably the spleen becomes infected before the liver.

Monkey D died on the 65th day from a mixed infection of tuberculosis and Leishmaniosis and it is interesting to record the degenerate nature of the parasites in this case. The parasites were most degenerated in the spleen, which was heavily infected with tubercle, and were best preserved in the bone-marrow where the tubercles were scanty. The post mortem examination was done immediately after death, so the change could not be a post mortem one.

Monkey E was infected by the 54th day, became very ill with diarrhœa on the 143rd day, and was chloroformed on the 145th day. It was heavily infected with parasites.

Monkey N, inoculated intraperitoneally from Monkey E, remained quite well, but, owing to infection occurring in Monkey S (*vide infra*), liver puncture was carried out on the 120th day and doubtful Leishman-Donovan bodies were seen; 35 days later the monkey died and the post mortem examination showed splenomegaly with Leishman-Donovan parasites present in the spleen, bone-marrow and liver. There was a small ulcer in the small intestine, in the region of the ileum.

Monkey P died on the 134th day heavily infected with Leishman-Donovan parasites.

Infection by a subcutaneous injection

Of those infected subcutaneously, Monkey R was inoculated in the subcutaneous tissues of the thigh with a post mortem splenic emulsion from a case of kala-azar. On the 150th day the spleen was found to be enlarged and liver puncture showed the presence of Leishman-Donovan bodies. On the 156th day the peripheral blood was examined and in the 5th slide 3 parasites were found inside a large mononuclear cell. This monkey is still alive.

Monkey L was injected intravenously and subcutaneously. It was intended to inject intravenously, but only the first portion of the virus entered the vein, the remainder entering the subcutaneous tissues of the forearm. Examined on the 161st day the spleen was found to be enlarged and splenic puncture showed the presence of Leishman-Donovan bodies. This monkey is still alive.

The remaining monkey, which was a very young animal, was put in the same cage as Monkey N on the day that the latter was inoculated intraperitoneally from Monkey E.

It remained continuously in that cage, and on the 112th day it became ill. On the 117th day liver puncture showed the presence of Leishman-Donovan bodies. It died on the 121st day, 34 days before Monkey N. It was very heavily infected, the parasites being present in large numbers in the liver, spleen and bone-marrow.

Non-infected cases

Non-infected cases

Monkey H was inoculated with a splenic emulsion from Case I., the emulsion being made 8 hours after the death of the patient, when no parasites were present in the spleen smears. (Monkey E was successfully infected from the same case during life). It was chloroformed on the 65th day, but no Leishman-Donovan parasites were found.

Monkey C was inoculated with a post mortem spleen emulsion from Monkey B, but was not infected by the 108th day, when it was chloroformed. Probably, as Nicolle has pointed out, the parasite tends to lose its virulence when inoculated from monkey to monkey, and this might account for the non-infection in this case, though the positive results obtained in Monkeys L and N show that infection can be conveyed from monkey to monkey. In the two latter, however, the inoculation was made from a splenic puncture during life, and in this monkey a post mortem splenic emulsion was used.

Monkey D died 42 days after inoculation. There was no apparent cause of death, and no Leishman-Donovan parasites were found.

Monkey K, which was inoculated subcutaneously, shows definite enlargement of the spleen, but one liver puncture and two spleen punctures, have so far given negative results.

The presence of Leishman-Donovan parasites in the blood of infected monkeys

The presence of Leishman-Donovan parasites in the blood of infected monkeys

Parasites are present in the peripheral blood of infected monkeys, but are difficult to find. In two infected monkeys we have searched carefully for parasites in the peripheral blood. In one monkey we found, in the fifth slide examined, three parasites inside one large mononuclear cell, and in another monkey, out of 7 slides examined, we found one parasite. In this instance it was inside a polynuclear cell.

Though dogs have been more frequently used in experimental kala-azar, Nicolle and his colleagues have frequently infected monkeys, though so far only by the intraperitoneal method, the subcutaneous method giving only a local reaction, without a general infection. They also found that passage of the disease from monkey to monkey attenuated the virus, though the same phenomenon did not apply in dogs. They consider that in the monkey the disease more resembles the human type, marked enlargement of the spleen being usually present. Our results agree with those obtained by these observers, but we have succeeded in infecting monkeys by subcutaneous inoculation, and have also naturally infected one monkey.

Experimental Kala-azar in the Dog

Inoculation experiments were also carried out to determine if the dog could be infected with kala-azar and the results are shown in Table II. (*page* 166).

TABLE II

Animal	Where inoculated and date of inoculation	Material inoculated	Course of Disease	Result	Organs infected	Remarks
Dog B ...	Liver and peritoneum March 12, 1910	Post mortem splenic emulsion Monkey B	14th day Died	Not infected	Spleen – Liver – Peritoneal fluid –	
Dog D ...	Liver and peritoneum May 27, 1910	Spleen puncture Case 1	31st day Liver puncture negative 50th day Axillary abscess Emaciation Liver puncture negative 52nd day Died	Not infected	Liver – Bone-Marrow –	Spleen smears unsatisfactory Post mortem examination some hours after death
Dog F ...	Peritoneum July 2, 1910	Liver puncture Monkey E	16th day Again inoculated intraperitoneally Spleen puncture Monkey E 19th day Died	Not infected	Spleen – Liver – Bone-Marrow –	
Dog G ...	Subcutaneous tissues July 2, 1910	Ditto	16th day Ditto 22nd day Died	Not infected	Spleen – Bone-Marrow –	
Dog H ...	Peritoneum September 13, 1910	Post mortem spleen emulsion Monkey E	7th day Died	Not infected		Piroplasmosis present

Infection of dogs was, therefore, not obtained, but it is difficult to account for the mortality. Probably the mortality in Dogs F and G, which were re-inoculated on the 16th day with the contents of a splenic puncture (Leishman-Donovan parasites not being seen in smears made from the original liver puncture with which they were inoculated), may be attributed to some anaphylactic effect of the body fluids from Monkey E. Dog D became emaciated, developed an abscess in the axilla, and we thought it would be infected, particularly as the spleen was found enlarged after death. Unfortunately, the post mortem examination was not done until some hours after death, when the spleen pulp was too soft for satisfactory examination, but the bone-marrow contained no parasites. As Dog D was the only one which lived long enough to become infected with the disease, further experiments are necessary to determine if the dog can be experimentally infected with the Sudan form of kala-azar.

Kala-azar in the dog

A large amount of experimental work has now been done on kala-azar in the dog. Nicolle and his colleagues have frequently infected dogs. They find in dogs that the infection is an insidious one with only slight enlargement of the spleen. One of the dogs which they inoculated with the disease and which died 17 months after inoculation showed no symptoms for 15 months. They also found that in a series of dogs there was great variation in the susceptibility of the individual animal. They found no attenuation in the virus by passage from dog to dog. With regard to immunity they have found that in an animal incompletely cured or only just recently recovered, a second inoculation produces a severe and fatal result, whereas, in an animal cured for some time immunity is conferred. Excision of the spleen in the dog has produced no effect on the course of the disease.

Jemma, Gabbi, and Alvarez and Pereira da Silva, have also all succeeded in infecting dogs intraperitoneally with *Leishmania infantum*. Basile was able to infect three young dogs by keeping them beside cases of infantile kala-azar and he thinks the flea (*Pulex serraticeps* and perhaps *Pulex irritans*) is the likely transmitting agent. Jemma has also been able to infect dogs intravenously as well as intraperitoneally.

Novy, by the use of large and repeated doses of cultures of *Leishmania infantum* on Novy and MacNeal's medium, has been able to produce infection in the dog.

Patton, working with Indian kala-azar, failed to infect dogs even with repeated inoculation, but probably he killed his animals too soon to say definitely that infection does not occur.

Morphology and Culture of the Parasite

The parasites in these cases do not appear to differ in structure from the parasites described in Indian kala-azar and in infantile kala-azar. The parasites were sometimes present in spleen cells and sometimes lying free. So-called developmental forms were frequently found containing up to as many as 11 nuclei. Our observations agree with those of other observers who find that, when a large amount of blood is obtained in spleen puncture, the parasites are scanty and difficult to find. There is definite evidence that the parasite degenerates quickly after death. As shown above, in one case where the parasites were found in large numbers during life the post mortem examination, 8 hours after death, showed no parasites in the spleen. In another case, examined 4 hours after death, the parasites were present but showed evidences of degeneration, the nucleus and kinetonucleus being visible, but no protoplasm.

Rapid degeneration of the parasites after death

In two cases the parasites were successfully cultivated in 10 per cent. citrate. Flagellated forms were found as early as the second day, but seemed to be most numerous and most active on the 4th and 5th days. The parasites move very slowly with the

flagellum in front. The flagella are usually very active. There are other forms present, some very large, which show no tendency to develop into the flagellate form. These measure about 5μ by 6·5μ, but we saw one measuring 7·65μ by 6·8μ. We have found living parasites in these cultures on the 28th day.

Culture

The parasite also grows on Novy and MacNeal's medium and on Nicolle's modification of that medium. We obtained cultures on these media with the material from the splenic puncture of an infected monkey.

Cultures made from post mortem spleen punctures were invariably negative, even though the parasites were present in considerable numbers.

Infection by the Bed-Bug (*Cimex lectularius*)

Feeding experiments negative

Feeding experiments with the bed-bug (*Cimex lectularius*) were carried out in Cases 1 and 3, but with negative results. So far, we have not met with the *Cimex rotundatus* in the course of our investigations. Owing to the small number of parasites found in the peripheral blood in our cases, these feeding experiments are of little value, as, in cultural experiments of the parasite, we found that the presence of a considerable number of parasites was necessary to give a positive growth into flagellate forms.

Other Protozoa met with in the course of the Investigation

(*a*) Protozoon from post mortem splenic puncture of Case 4.
(*b*) Flagellated bodies found in water and in fæces of infected monkey.
(*c*) Flagellated bodies present in soil.

(*a*) *Protozoon from post mortem splenic puncture of Case* 4 (Plate X., figs. 5–7)

Cultures were made from the post mortem splenic puncture of Case 4 from which Monkey B was successfully infected with kala-azar. After 48 hours the four culture tubes were examined; three showed micro-organisms, the fourth showed micro-organisms and unaltered Leishman-Donovan parasites. Examination of this tube, 4 days later, showed the presence of large, slightly motile bodies with a tendency to become oval and, evidently, from the currents produced, ciliated. They appeared to possess amœboid properties. These bodies developed, in a short time, into oval, actively motile bodies moving always with the sharp end in front. In a hanging-drop slide ringed with paraffin they remained motile for at least 48 hours. A contractile vacuole was visible at the posterior end. Stained preparations show a definite pale area near the anterior end, sometimes on the right side and sometimes on the left. This has the appearance of a peristome. These bodies are ciliated all round but the cilia are more numerous at the anterior end, and are particularly so round the pale area. The nucleus is large but varies in shape and situation. The protoplasm is vacuolated and full of bacteria. Amœboid projections from the edge of the body are sometimes present, a small projection from the posterior end being particularly common.

Subculture unsuccessful

This parasite appears to live indefinitely in the original fluid, though subculture on various media always gave negative results, the parasite not being able to multiply and soon dying out. Injected intraperitoneally, it was non-pathogenic for a young dog. The parasite was able to live for some days in media containing blood, but did not multiply. The younger stage of this parasite is seen as a small, circular, deeply-stained nucleated body occurring for the most part in groups. Whether these

are the result of encystment and rupture, or whether they are budded off from the parent body, we cannot tell. These small round bodies develop into small oval bodies which gradually increase in size, becoming in time a full-grown parasite. The size of a full-grown parasite is about 42·5 μ by 34 μ.

This parasite evidently belongs to the *Balantidium* group of protozoa, but does not conform to the description given of either of the two described as occurring in the human alimentary canal. It is smaller than *Balantidium coli*, and differs also in having only one nucleus and one contractile vacuole. It is bigger than the *Balantidium minutum* of Schaudinn, and differs also in the structure of the peristome. The special aggregation of the cilia at the anterior or pointed end is also characteristic of this new form.

Balantidia are usually associated in man with pathological conditions, producing enteritis and sometimes ulceration of the bowel. It is an interesting fact that it was obtained by splenic puncture from a case of kala-azar which had suffered from diarrhœa. It is also interesting to record that this parasite was again obtained in a culture made from the mucous membrane around the ulcer in the large intestine of Case 11.

(*b*) (1) *Flagellated bodies in standing water* (Plate X., figs. 8 and 9)

Flagellated bodies in standing water

This parasite was found accidentally while we were endeavouring to cultivate flagellated bodies from the fæces of infected monkeys. The control of ordinary distilled water showed in three to four days an enormous number of very actively motile bodies. These bodies are small, oval or circular, and, in stained preparations, appear to have usually two flagella. The longer flagellum comes from the region of the nucleus and the shorter one from the kinetonucleus. The protoplasm is vacuolated. The average measurement is 6·8μ by 5μ. The flagellum is usually longer than the parasite. On examining the original bottle containing the distilled water a few parasites were found. These evidently multiply rapidly at 22° C.

(*b*) (2) *Flagellated bodies in fæces of infected monkey* (Plate X., figs. 10–12)

Flagellated bodies in fæces of infected monkey

These were obtained by emulsifying the fæces of Monkey E in boiled distilled water and incubating at 22° C. These bodies very much resemble the preceding, but are smaller and more elongated. Sometimes one flagellum was present and sometimes two, the latter being the more common. Usually the two flagella arise quite close together, and, in many parasites, the kinetonucleus is not visible. The average length of the parasite is about 6μ by 3μ. Further experiments are necessary to determine if these parasites are present in the fæces of other infected monkeys, controls with healthy monkeys so far having given negative results.

(*c*) *Flagellated bodies in soil*

Flagellated bodies in soil

Enormous numbers of flagellated bodies were obtained from soil, but their morphology has not yet been worked out.

Conclusions

1. Kala-azar in Sennar Province affects, for the most part, children whose average age is about 12 years. This does not appear to hold in the neighbouring Province of Kassala where adults are commonly affected.
2. The disease runs an acute course, and chronic cases were not met with.
3. The disease is not invariably fatal, cases of recovery having been met with.

4. The Leishman-Donovan parasite was found in the peripheral blood in 86·6 per cent. of the cases.
5. The ordinary grey monkey of the Sudan (*Cercopithecus sabæus*) can be infected with kala-azar.
6. It can be infected by intraperitoneal inoculation and by subcutaneous inoculation.
7. Natural infection, from an infected to a healthy animal, can occur provided the animals are kept in close contact.
8. The Leishman-Donovan parasite is present in the peripheral blood of infected monkeys.
9. So far we have found that the best method of infecting the monkey is by injecting into the peritoneal cavity the contents of a spleen puncture taken during life.
10. So far we have not succeeded in infecting dogs nor have we found spontaneous Leishmaniosis in the dog.
11. The parasite can be cultivated readily on artificial media, development into flagellate forms being obtained in 10 per cent. citrate, on Novy and MacNeal's medium and on Nicolle's modification of that medium.
12. The parasite appears to degenerate quickly after the death of the host. Post mortem examinations and post mortem spleen punctures for diagnostic purposes should be done immediately after death.

Possible Methods of Infection in Kala-azar

From the evidence obtained it appears likely that some insect acts as the transmitting agent of kala-azar in the Sudan. You can take unaltered parasites from one monkey, inject them subcutaneously into another monkey, and the latter becomes infected with the disease, so that presumably the disease can be conveyed without any extracorporeal development into the flagellate stage. Further, a healthy monkey kept beside an infected monkey can contract the disease, and in the case recorded in this paper the infection was a rapid and fatal one, and was contracted from a monkey which was not heavily infected with the disease. How then did this monkey become infected? If an insect, the bed-bug (*Cimex lectularius*) is unlikely, as these monkeys were kept in a metal cage and no bed-bugs were found, so that some other insect transmitter must be looked for, and further experiments will be carried out to elucidate this point. Possibly also, if an insect is the transmitting agent, only a small percentage of the feeding or biting animals become infected (owing to the small number of parasites in the peripheral blood) and this would account for the absence of epidemics and the apparently wide-spread distribution of the disease. The presence of a flagellate form also suggests an intermediate host and a developmental stage outside the human body.

Probability of an "insect" vector

The other possible source of infection is an intestinal one, the flagellate form occurring free outside the body and entering the body again by water or by food. If so, in the naturally infected monkey, the monkey must have eaten food contaminated by the excreta of the infected monkey.

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The Alkalinity of the Blood Serum in Kala-azar

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The recent researches of Nierenstein[1] on the acidity and alkalinity of the blood in trypanosomiasis infections suggested the idea that a similar line of researches might be carried out in kala-azar, with the object of ascertaining if any change occurred in the blood serum of patients suffering from this disease.

Owing to the fact that kala-azar is still a somewhat uncommon disease in the Sudan, it was difficult to obtain sufficient material for an extensive number of tests, and although these results were obtained from only four undoubted cases of kala-azar, they are published in the hope that some specific line of treatment may be adopted in accordance with the changes that have been found to exist in the blood serum of patients suffering from this fatal disorder.

The tests were carried out in patients in whom the presence of the Leishman body had been previously demonstrated by the microscope.

The technique employed was that recommended by Moore and Wilson,[2] and the tests were conducted on the "entire serum" in preference to the whole blood, as Wright[3] has pointed out that from a clinical point of view the alkalinity of the serum is more important as it comes into such close contact with the tissues, and may be taken as an index of the changes taking place in the circulating blood. Formerly, litmus was usually employed as an indicator for estimating the alkalinity of the blood, but Moore and Wilson found that sharper readings of the colour changes could be obtained by the use of dimethylamidoazobenzol, and accordingly this was employed in the tests.

The "entire serum" used for tests

Technique.—The blood was collected in Wright's glass capsules, samples being taken from the finger previously cleansed with methylated ether and 5 per cent. formalin. The ends of the capsules were sealed in the ordinary way, the tubes left suspended for twenty-four hours, and then centrifuged so that the serum separated out free from the red cells. If the serum was stained as a result of hæmolysis, it was always discarded.

In each experiment a "control" was used, the blood serum being taken from three healthy persons, whose ages and racial characters corresponded as nearly as possible with those of the patient. In order to avoid the effects of an "alkaline tide," the blood from the patient and from the controls was always secured four hours after the last meal had been taken.

The control

Normal sulphuric acid was employed as the standard acid for titration, and a series of dilutions of the normal acid were made, ranging from a twofold to a sevenfold dilution.

To every 100 c.c. of these acid dilutions were added 8 drops of a 1 per cent. alcoholic solution of dimethylamidoazobenzol, a rich pink colour, sufficiently intense to show the necessary colour changes when mixed with serum, being thereby produced.

[1] Nierenstein, M. (1908), "Observations on the Acidity and Alkalinity of the Blood in Trypanosome Infections." *Annals of Tropical Medicine and Parasitology*, Vol. II., No. 3.

[2] Moore, B., and Wilson, F. P. (June, 1906), "Clinical Method of Hæmalkalimetry." *Biochemical Journal*, Vol. I.

[3] Wright, A. E. (1897), "A Simple Method of Measuring the Alkalinity of the Blood." *Lancet*, Vol. II., p. 719.

The same amount of indicator was always added to the acid solutions, and when ready for use the mixture of acid and indicator was poured into a watch-glass.

Titration of serum against the standard acid solution.—The blood capsules, after centrifuging, were nicked with a file, and the end nearest the serum broken off. The sera of the control individuals was then "pooled" and placed in a separate glass capsule.

A fine capillary pipette, such as is used for estimating the Widal reactions, was inserted into the serum, and a column of serum allowed to run up to a mark made with a blue pencil, about 2 cm. up the tube. The pipette was then withdrawn, a small bubble of air sucked in, and the pipette inserted into the acid until the upper column of the acid was level with the blue mark. In this way equal volumes of serum and acid of a known strength were obtained.

The contents of the capillary tube were then blown out on a clean white slab, thoroughly mixed with the end of the pipette, and aspirated in and out of the capillary tube several times.

The reading of results

In doing this it was found advisable to avoid the presence of air bubbles in the mixture. If a yellow-coloured fluid resulted, a stronger acid was taken until an orange-red was given, and the dilution of acid which last gave this yellow colour was taken as the index.

If, on the other hand, a pink or orange colour resulted from the first titration, then a weaker acid was used till a yellow colour, free from red, was obtained, and the first dilution of acid which gave this yellow colour constituted the index. As far as possible, all the following estimations were carried out under similar conditions.

All glass tubes, pipettes, and flasks used throughout these experiments were subjected to immersion in pure hydrochloric acid for half an hour, washed in distilled water several times, and then dried in an oven at 130° C., in order to drive off any residual acid. This precaution was taken for the purpose of avoiding any fallacious results due to the presence of alkali in the glass. After this treatment, distilled water left in the tubes for twenty-four hours gave neither an acid nor an alkaline reaction.

Case 1.—An Egyptian soldier, aged 30, with a previous history of irregular fever of two months duration. The general condition of the patient was fair. Several blood examinations failed to show the presence of the parasite of kala-azar. The spleen was enlarged. Splenic puncture showed the presence of Leishman bodies. Soon after diagnosis, and before the patient was placed on any specific line of treatment, the reaction of the blood serum to "dimethyl" was tested, and the result was as follows :—

Pooled normal serum	...	...	0·033 expressed as a fraction of normal
Patient's serum	...	...	0·025 ,, ,, ,,

Alkalinity diminished

This was, comparatively speaking, not a very advanced case, but the alkalinity of the blood of the patient, when compared with the pooled serum, will be seen to be diminished.

Case 2.—An Egyptian soldier, about 28 years of age, with a previous history of recurrent attacks of fever when stationed in the Blue Nile Province. Patient was admitted in a weak condition with a high fever and enlarged spleen. Examination of a peripheral blood film failed to show the presence of malarial or other parasites. The spleen extended to about an inch below the umbilicus, and the lower limit of the liver could be palpated below the costal margin. Splenic puncture showed Leishman bodies to be present.

The serum reaction was tested and showed a diminished alkalinity :—

Pooled normal serum	0·03	expressed as a fraction of normal
Patient's serum	0·024	,, ,, ,,

Case 3.—An Egyptian soldier, about 30 years of age. He was admitted into hospital with a previous history of fever in the Kassala Province.

The general condition of the patient was fairly good. Both spleen and liver were enlarged, but the former was not sufficiently enlarged to justify splenic puncture. Repeated examinations of the peripheral blood having given negative results as regards the presence of the malarial parasite, the liver was punctured and a few Leishman bodies were found to be present. The alkalinity of the patient's serum was estimated two days after the diagnosis had been confirmed, and the results were as follows :—

Pooled normal serum	0·035	expressed as a fraction of normal
Patient's serum	0·023	,, ,, ,,

There was obviously a marked diminution in the alkalinity of the patient's serum.

Case 4.—A Sudanese boy, aged 14, a native of the Blue Nile Province, admitted to hospital suffering from epistaxis and diarrhœa. The previous history was very indefinite. On admission, a history of epistaxis since childhood was obtained. Patient was somewhat thin and cachectic, and when lying in the prone position an abdominal swelling was apparent. This, on palpation, proved to be an enlarged spleen extending downwards 1 inch below the umbilicus. The liver was also enlarged. Examination of the peripheral blood was negative as regards the presence of malarial parasites, and splenic puncture was resorted to as an aid to diagnosis. Examination of the splenic smears revealed the presence of a large number of Leishman bodies.

The alkalinity of the patient's serum was tested soon after the diagnosis had been confirmed, and was as follows :—

Pooled normal serum	0·033	expressed as a fraction of normal
Patient's serum	0·020	,, ,, ,,

A blood count and an estimation of the hæmoglobin were undertaken in this case, and the figures were as follows :—

Red blood corpuscles	2,916,000
White blood corpuscles	4,680
Hæmoglobin	60 per cent.

A differential count showed :—

Polymorphonuclear leucocytes	43 per cent.
Large mononuclears	32 ,,
Lymphocytes	23 ,,
Transitionals	1 ,,
Eosinophiles	1 ,,

The patient was treated, soon after admission, with increasing doses of *liquor arsenicalis* for a period of one month, and during this period little change was noted in his general condition.

Clinical application of the findings

In view of the fact that a diminution in the alkalinity of the serum was found, and that such clinical signs and symptoms in kala-azar as epistaxis, ulceration of the buccal mucosa, petechiæ, etc., are common features in scurvy, Dr. Balfour suggested that an alkaline treatment might be efficacious.

Accordingly, calcium lactate in 30-grain daily doses was given, and in order at the same time to obtain, if possible, a direct lethal action on the parasite, orsudan in 1-gramme doses was administered intramuscularly four times a week.

The calcium lactate was given in increasing doses until the patient was taking 50 grains *per diem*. The spleen was found to be diminishing in size and the patient's general condition improving slightly. The diarrhœa and epistaxis ceased.

After three weeks' treatment the spleen had retracted to 1 inch above the umbilicus.

The patient's serum alkalinity was again tested and found as follows :—

Pooled normal serum	0·03	expressed as a fraction of normal
Patient's serum	0·026	„ „ „

The alkalinity of the patient's serum had evidently increased after a month's treatment with large doses of calcium lactate, and the general condition of the patient had improved ; but judging from the blood count and the hæmoglobin percentage, it was apparent that the prognosis was extremely bad. The count showed as follows :—

Red blood cells	2,666,000
White blood cells	3,125
Hæmoglobin	50 per cent.

A differential count showed :—

Polymorphonuclear leucocytes	32	per cent.
Large mononuclears	44	„
Lymphocytes	21	„
Transitionals	2	„
Eosinophiles	1	„

In addition to the calcium lactate, hæmatinics were also administered, but at the end of a fortnight the patient relapsed.

Two days previous to death the hæmoglobin percentage had fallen to 25 per cent.

Post mortem appearances

The post mortem appearances of the spleen and liver were typical of kala-azar, both organs being enlarged and the liver cirrhotic. Smears revealed numerous Leishman bodies. Ulcers were not present in the intestines. The mucosa of the small intestine was very anæmic and considerably atrophied, and resembled the consistency of thin tissue-paper. This latter condition has been present as a constant pathological condition in almost all the post mortems of these kala-azar cases. With such an atrophic condition of the mucosa of the small intestine the physiological functions of this portion of the gut are practically in abeyance, and consequently the emaciation of the patient progresses steadily in spite of all efforts to combat it.

Conclusions

It is evident that the alkalinity of the blood in these four cases of undoubted kala-azar was diminished, and it is worth remembering that Rogers, Leishman, Statham, and other observers found that the ideal culture medium for successful cultivation of the Leishman body is an acid one.

If, then, the alkalinity of the blood serum in cases of kala-azar be diminished, it is conceivable that the blood and tissues present a more favourable medium for the Leishman parasite than is usually the case; and, in order to obviate this, it would not seem unreasonable, whatever specific line of treatment be adopted, that drugs should be administered which tend to increase the alkalinity of the blood.

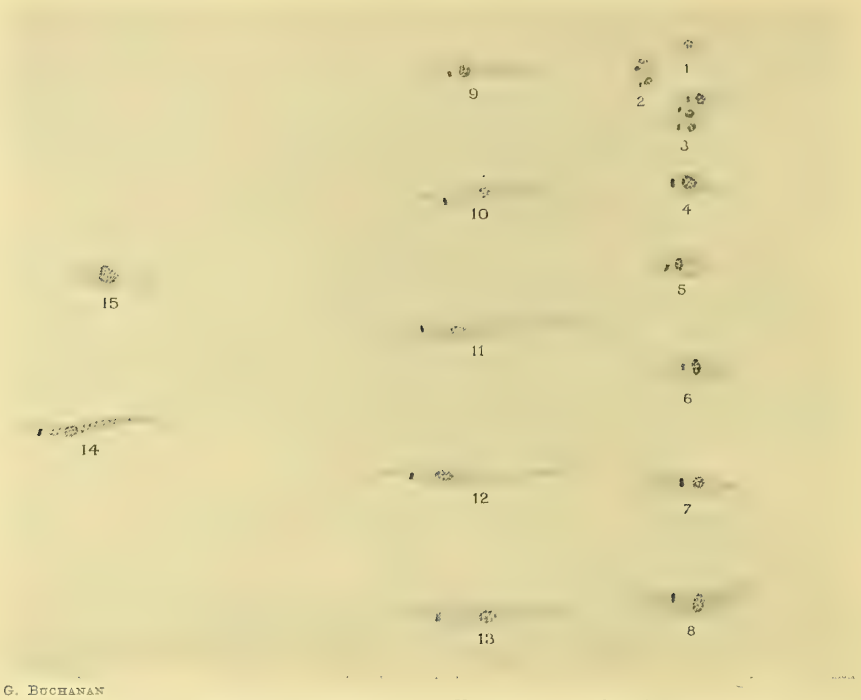
Of the above cases, only one was subjected to an alkaline treatment by calcium lactate, and unfortunately the disease was so far advanced in this instance that the drug had scarcely a fair trial.

A specific drug for the successful treatment of Leishmaniosis has not yet been found. Quinine and various arsenical preparations have not given satisfactory results up to the present,[1] and hence the record of these four cases points to the advisability of employing such remedies as would increase the alkalinity of the blood.

Further, the alkaline reaction obtained in testing the serum of kala-azar cases may be of service in assisting the diagnosis in early cases where splenic enlargement is not marked.

I am indebted to Colonel Mathias, D.S.O., Principal Medical Officer, Egyptian Army, for his kind permission to publish these results, and to the officers of his staff at the Khartoum Military Hospital, for their assistance.

[1] Muir has recently reported favourably on the use of quinine by hypodermic injection, especially in early cases.—*Indian Medical Gazette,* February, 1911.—A.B.

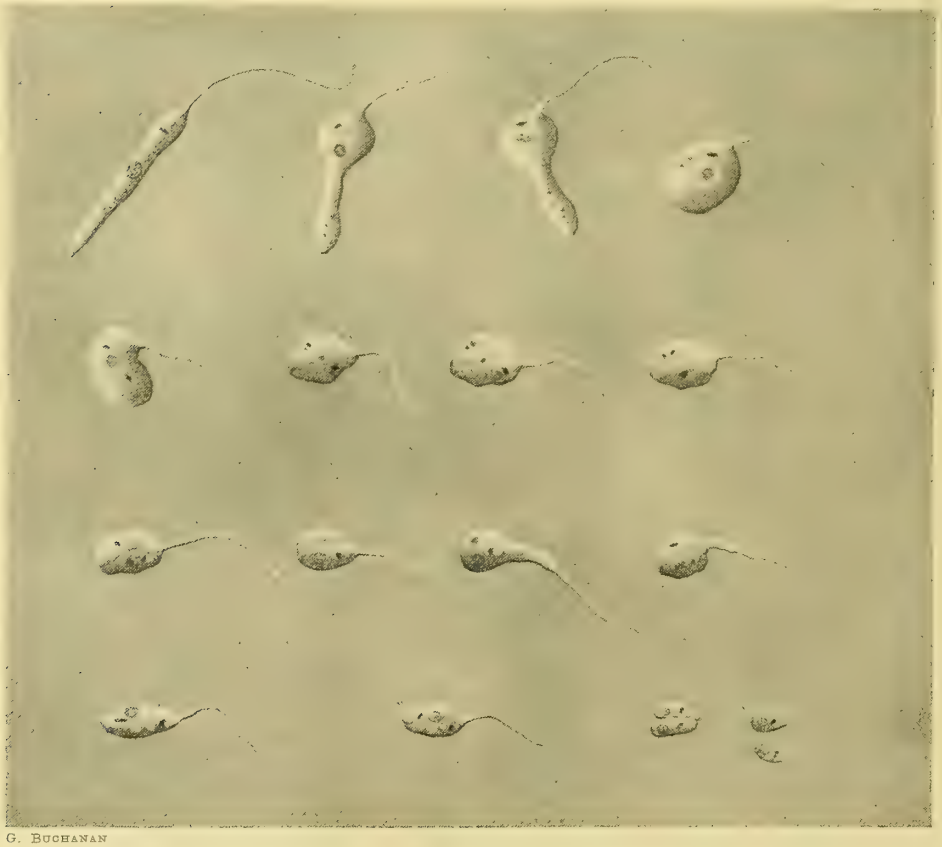


G. Buchanan

FLAGELLATE FORM OF *Herpetomonas lygæi*

1–6. Bodies resembling the Leishman-Donovan parasite. 7–9. Intermediate stages.
10–14. Fully-formed stages. 15. Form without visible kinetonucleus.

× 750 *diam.*



G. Buchanan

16. DIAGRAMMATIC ILLUSTRATION OF CYST FORMATION OF *Herpetomonas lygæi* IN THE HIND GUT OF *Lygæus militaris*, AS SEEN IN A HANGING-DROP PREPARATION

× *approx.* 1000 *diam.*

Herpetomonas lygæi

Considered from a Protozoological and Medical Aspect

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The relationship existing between the genera Trypanosoma, Herpetomonas, Crithidia and various diseases which affect man, is still somewhat in a state of chaos. Some of the parasites of these genera are known to have as their hosts various insects and biting flies, but the rôle played by these as transmitting agents of such diseases as Kala-azar, Trypanosomiasis, and Oriental Sore still requires further elucidation. While I was engaged in studying a certain type of Herpetomonad which was found to infest the alimentary tract of the dura plant bug in the Sudan, a paper was published by Patton[1] describing a similar type of flagellate which is parasitic in the *Lygæus militaris* found in India. The same insect, known as the dura plant bug, is also ubiquitous in the Sudan, and a rich material enabled one to study in detail the morphology and life-cycle of the parasite infesting its intestinal tract. *Lygæus militaris* is a brilliantly coloured hemipteron* which exists as a pest in various parts of the Sudan, doing considerable damage to the dura or millet crops of the country. Its ravages, however, are not confined to dura alone, for it has been found feeding on various plants, notably the *Calotropis procera*. By means of a piercing apparatus it punctures the plant and destroys its vitality by withdrawing the sap.

Characters of *Lygæus militaris*

If the alimentary tract of this bug be dissected out, it will be seen to consist of a very short œsophagus opening into a large sacculated crop in which peristaltic movements can be observed taking place. The crop opens into a narrow and long mid-intestine which ends in a bulbous dilatation. At the other end of this dilatation a short ileum extends posteriorly and connects with a dilated colon in which are found the openings of the Malpighian tubes. From the colon, the remainder of the alimentary tract is represented by a short tube opening into the rectum.

Material and Methods Employed

In order to study the different stages of the flagellate in the various portions of the alimentary tract of *Lygæus militaris*, the method employed, and one which gave satisfactory results, was to dissect out the alimentary tract of the bug in normal saline solution and then to transfer the various portions, with their contents intact, to several watch-glasses containing fresh normal salt solution, where emulsions were made and transferred to slides, spread, and then stained in a suitable manner. Fresh hanging-drop preparations were also made in order to study the various stages of the flagellate.

A wet method of fixation was usually employed before the films were stained, Leishman's modification of Romanowsky or iron hæmatoxylin being the stains used. The latter was chiefly used to differentiate the nuclear and chromatic structures present in the herpetomonad.

Fixation of films

[1] Patton, W. S. (1908), *Herpetomonas lygœi. Arch. f. Protistenk.*

* *Vide* Second Report of these Laboratories, fig. 56, *p.* 95.

Patton in his paper described the life-cycle of this herpetomonas as occurring in three different stages which he names the preflagellate, flagellate and post-flagellate. All these stages can be respectively seen in various parts of the alimentary tract of *Lygæus militaris*.

If a hanging-drop preparation of the crop contents be examined, adult flagellates can be observed, but not in very great numbers. They appear to be more plentiful in the small mid-intestine.

Activity of the flagellates

They are actively motile and display various types of movement, the commonest of which is a jerky, wriggling motion, with the flagella end forward, the latter vibrating vigorously; this movement is often followed by a peculiar gliding one in which the flagellum is in a state of quiescence, standing out from the body in a straight line. At other times the flagellum displays lashing movements, and occasionally the herpetomonad moves forward by means of its posterior end, while the flagellum trails behind. When the latter becomes attached to any object the body appears to bend, and sways to and fro in a rhythmical manner, at the same time twisting on itself. The twist may occur both ways from right to left and vice versa. Sometimes the flagellate appears to rotate on its own axis showing a peculiar action resembling the movements of a "Catherine wheel." All these movements are brought about by the presence of myonemes in the body of the flagellate. Waves of contraction pass along these body myonemes from the posterior to the anterior end. These waves of contraction can be well seen in the herpetomonad just prior to its encystment, when its movements become very sluggish.

As each wave of contraction passes along the body, small refractile granules can be seen alternately advancing and receding down the flagellum for about a third of its length.

The Preflagellate Stage

Plate XI., figs. 1–6

If the crop contents be examined for the presence of cysts, very few will be found; this is due to the fact that such a rapid development of the cyst occurs in the crop that practically only flagellates are found. The early cystic stage is really best studied in the fæces where they are more numerous.

The preflagellate stage in Leishman-stained specimens are small, oval, pyriform or round cells, $2-4\mu \times 1-1{\cdot}5\mu$, with a pale blue cytoplasm, limited by a sharply defined edge or periplast. The pink trophonucleus is most often situated at the rounded end of the cell, and is either circular or bean-shaped, measuring about 1μ. When stained by iron hæmatoxylin, and suitably differentiated, a distinct nuclear membrane is apparent, but at this stage of the parasite chromosomes are rarely seen.

The kinetonucleus occupies a position anterior to the trophonucleus, and appears as a dark staining rod or dot in the more pointed or anterior portion of the cell. Vacuoles are occasionally to be found in the cell cytoplasm, and at this stage the flagellum may appear arising from the vicinity of the kinetonucleus and reaching the exterior as a pink streak.

Development—first stage

The first stage in the further development of the parasite is a general enlargement both in length and breadth, and the cell cytoplasm appears very markedly reticulated. The trophonucleus enlarges in size, and may occupy half, or more than half, of the cell area. The parasite, instead of appearing as a short compact little cell, now resembles a delicate structure more or less pear-shaped and measuring about 6μ in length. A clear area now begins to form round the kinetonucleus, which at this stage begins to elongate,

and shows a slight constriction in its middle, producing a dumb-bell like appearance. Division of the kinetonucleus then occurs, followed by division of the trophonucleus, and longitudinal division of the cell wall and its cytoplasm, and two separate parasites, each containing a tropho- and a kinetonucleus, are thus formed. The anterior ends of the parasite are usually directed towards one another or lie in close apposition. Each parasite may again go through the same process of division, and four or more parasites be thus formed. The further stage of development continues in the crop. Most of these parasites pass into the stage of flagellation while others may yet divide again before becoming flagellates. In those parasites that undergo further development, the anterior end becomes elongated and the kinetonucleus moves farther from the trophonucleus. This is followed by elongation of the posterior end of the parasite and the development of a vacuole anterior to the kinetonucleus; from this vacuole a pale pink filament can be seen developing. This represents the flagellum (Plate XI., figs. 7–9). Longitudinal division may then occur, the line of cleavage usually commencing at the anterior end of the parasite. Many of the parasites at this stage show marked vacuolation, especially at their posterior extremity, and appear as short stumpy tadpole forms with thickened kinetonuclei and flagella. The trophonucleus is often stretched out with its long axis running transverse to the parasite. All these various stages of development up to mature flagellates may be seen in the crop; the mid-intestine, however, shows the largest number of typical adult flagellates. These adult flagellates measure on an average 26μ in length and 2μ in breadth. In appearance they are somewhat cigar-shaped, with sharp pointed posterior ends, and blunt rounded anterior ends from which the flagella extrude. The cytoplasm is vesicular in structure, and often contains pink-coloured granules, especially in the posterior portion of the parasite (Plate XI., figs. 10–14).

Further stages

If an iron hæmatoxylin stain be employed it is quite apparent that these granules are not of a chromatin nature, for the cytoplasm is stained very uniformly in this area, showing, perhaps, a slight reticular structure, but rarely anything that could be considered chromatic granules. It is difficult to say what these granules represent; in all probability they are of the nature of reserve foodstuff. Porter[1] is inclined to think they represent metabolic changes as they are usually present when the parasite is in a highly nutritive medium, but, when deprived of this medium, the granules present are but few in number. This view is supported by the fact that herpetomonads present in the gut of bugs that have been starved for a few days show practically no granules in their cytoplasm, while those herpetomonads removed from bugs that have been recently feeding have a marked granular cytoplasm. The trophonucleus is, as a rule, circular in shape and occupies more or less a central position in the parasite. Around its margin is a definite nuclear membrane containing chromosomes; these are more apparent in specimens stained by Giemsa than by Leishman's stain. In iron hæmatoxylin specimens the nuclear membrane is well brought out but no central karyosome is evident.

A nuclear membrane

The kinetonucleus occupies a position anterior to the trophonucleus, and is usually 4μ distant from it. In adult flagellates it is invariably rod-shaped, running transverse to the long axis of the parasite, and is placed about 4μ from the anterior extremity. The cytoplasm between the tropho- and kinetonuclei stains quite uniformly and shows no evidence of the spiral "Doppelfaden" described by Prowazek in *H. muscæ domesticæ*.

Immediately in front of the kinetonucleus is a small achromatic space, on the anterior margin of which there appears a slight thickening of the cell cytoplasm in the form of a small rod or dot. This is rarely seen except in films fixed by a wet method.

[1] Porter, A. (1909), "The life cycle of *Herpetomonas jaculum*," Leger. *Parasitology*, Vol. II., pp. 367-391.

Origin of Flagellum

The flagellum arises from this small dot or basal granule which, according to Minchin,[1] ought to be termed the blepharoplast. After its origin from the blepharoplast it occupies a median longitudinal position in the parasite and passes out of its anterior extremity as a fine tapering filamentous structure, stained a deep red colour. It varies in length, and in adult flagellates it is about twice as long as the body of the parasite.

When the flagellate reaches the adult stage, longitudinal division usually occurs and two flagellates result. Prior to division, enlargement of the tropho- and kinetonuclei takes place, the latter becoming thickened and a constriction appearing in its middle. A second small flagellum, arising close to the original one, then makes its appearance, and one is inclined to think that its origin is a separate one and that it is not due to a splitting of the original flagellum. Many observations on hanging-drop preparations were made, but it was impossible to come to any definite conclusion concerning this much debated point. With the appearance of the second flagellum the kinetonucleus divides transversely into two separate portions, and this is followed by a similar division of the trophonucleus. A line of cleavage first forms between the sites of origin of the two flagella and extends in a posterior direction along the body of the parasite; when completed, two parasites result. These again may undergo a second division, passing through more or less identical processes. The parasite, therefore, multiplies by longitudinal division. Many observations were carried out with hanging-drop preparations for the purpose of ascertaining whether conjugation ever took place, but in no instance was this ever detected. In stained preparations one was often inclined to think that parasites of different sexes were present. Frequently, large stout forms measuring 30 μ in length and 3 μ in breadth and often devoid of a flagellum were present; they were granular in structure and in Leishman-stained preparations it was sometimes difficult to make out any differentiation between trophonucleus and kinetonucleus. They stood out in marked contrast to the more attenuated typical forms of flagellates.

In view of the fact, however, that conjugation was never observed, one must look on these stout forms as degenerated parasites.

Post-Flagellate Stage

Plate XI., fig. 16

Cyst formation

This is characterised in the first place by the herpetomonads becoming attached to the lining epithelium of the colon, and if this portion of the gut be teased out on a slide and stained, large masses of flagellates will be seen fixed to the gut wall by means of their flagella, their posterior ends lying free. They appear to have undergone no change in structure from the typical flagellates found in the mid-intestine and ileum, and in order to study the various alterations occurring in the parasite prior to encystment, the following method was adopted: A large drop of fluid was taken from an emulsion made up of the contents of the colon and placed on a glass slide; a cover-glass was then placed on this drop, and the margin of the cover slip ringed round with melted paraffin so as to exclude the entrance of air; a suitable hanging-drop preparation was thus made, and no evaporation occurred. If such a preparation be examined, the flagellates will be seen to show active movements for two or three hours. Several things may happen; the parasite may undergo longitudinal division once more, or, what is more common, it becomes detached from the lining membrane of the gut and is a free-moving flagellate. After two or three hours its movements begin to get sluggish and finally its position on the slide becomes a fixed one with only rhythmical wave-like contractions passing along from the posterior end to the flagellum. During these

[1] Minchin, E. A. (1908), "Investigations on the development of Trypanosomes in the Tsetse fly and other Diptera." *Quarterly Journal Microscopic Science*, Vol. LII., pp. 159-260.

contractions refractile masses can be seen passing along the flagellum for a distance of 5 μ or 6 μ. As these movements become more sluggish, granules are seen collecting in the posterior portion of the parasite. The anterior portion then commences to enlarge and dilate and becomes quite globular in shape; this enlargement and dilatation extend back to the posterior portion of the parasite and a complete alteration in its whole contour is thus produced (Plate XI., fig. 16); what was previously a cigar-shaped body has now become an irregular shaped cell with the flagellum still attached. That portion of the cell from which the flagellum arises retains for a short time its original conical shape.

Division of the kineto- and trophonuclei

Division of the kineto- and trophonuclei then occurs, the respective divided portions going to opposite sides of the cell, and finally the latter splits by a line of cleavage passing from the anterior to the posterior end. The two cells lie adjacent to each other, the original one still retaining the flagellum. Before the original cell has divided a large number of vacuoles and granules appear, the latter showing very marked Brownian movement.

During the whole of this stage, which occupied about an hour, the flagellum remained practically stationary, and longitudinal splitting of it was never observed. At regular intervals the flagellum was measured. Its intra-cellular portion showed a distinct curve, and this was associated with a diminution in length of the extra-cellular portion. This diminution continued to go on till the flagellum had shortened by 4–5 μ—and one concluded that this shortening was due to withdrawal of the flagellum within the cell. After this preliminary withdrawal the flagellum underwent a change in appearance, became more hyaline and often showed at its terminal end a granule. Finally, it ruptured from the anterior end of the cell and was disintegrated. Further divisions of the cell occurred till oval or pyriform bodies, about 2 μ in length, were formed. These represented the cysts which were found in large numbers in the fæces of *Lygæus militaris*. These cysts resembled in appearance the cysts found in the crop and require no further description. This cystic stage concluded the life-cycle of the herpetomonas found in *Lygæus militaris*.

Method of Infection

The method of infection is brought about by the accidental ingestion of the cysts passed in the fæces; the latter are deposited on the surface of plants, and infection is thus brought about.

Infection of *Lygæus* not hereditary

The parasites were only found in the alimentary canal of *Lygæus militaris*. The ova of a large number of infected bugs were examined and in no case were herpetomonads found; so one concluded that infection is not transmitted by heredity. This was borne out by the simple experiment of removing the eggs, laid by an infected female bug, to a clean jar. In about a week the larvæ hatched out, and in the course of a month or so became adult bugs. These were dissected at various stages of their growth, and in no case did they show infection with herpetomonads.

Culture and Animal Experiments

Owing to the close similarity that exists between *Herpetomonas lygæi* and the Leishman body of kala-azar, it occurred to one that the relationship between the two was a closer one than their mere morphology suggested, and that the presence of the flagellates, found in the bug, might in some way account for a disease like kala-azar which exists in certain parts of the Sudan. Accordingly, a number of inoculations and cultural experiments was carried out.

Emulsions of the crop contents of a heavily infected bug were injected into the

No demonstrable connection with the parasite of Kala-azar or Oriental Sore

peritoneal cavity of a *Cercopithecus* monkey, a species of monkey which can be artificially inoculated with kala-azar. Examinations of the peripheral and splenic blood of this monkey were carried out at various intervals, but all with negative results, and as the monkey showed no clinical signs of kala-azar the result of this experiment may be considered a negative one. A second experiment was carried out in which the fæces, containing a heavy infection of herpetomonas cysts, were injected into the peritoneal cavity of another monkey. This experiment, like the previous one, yielded a negative result. Experiments on the same lines were carried out on rabbits and on one guinea-pig, and they also gave negative results.

Cultivation experiments were carried out on different culture media, the best results being obtained by using Nicolle's medium at a temperature of 25° C. In this medium, the flagellates lived and multiplied for a period of three days, but subsequently lost their motility and became disintegrated. A temperature of 37° C. did not appear a suitable one for their growth.

Quite recently, oriental sore has been found to exist in the Sudan, and, with the view of ascertaining whether any relationship existed between the parasites of that disease and those found in the gut of *Lygæus militaris*, the following experiment was carried out. The fæces and crop contents of a heavily infected *Lygæus militaris* were placed on the surface of an abrasion artificially made on the leg of a *Cercopithecus* monkey. Living flagellates were found to be present at the end of six hours, after which they became disintegrated, and, at the end of 24 hours, no trace of either flagellates or cysts were visible in the lesion. A further examination of this lesion, carried out a week later, was also attended with negative results.

Patton, in his researches on kala-azar in India, found that the Leishman body underwent development changes in *Cimex rotundatus*, and naturally concluded that this bug played a possible rôle as a transmitting agent of the disease. In the Sudan, *Cimex rotundatus*, until recently, had only been found on the Red Sea coast,* but the allied species, *Cimex lectularius*, is practically ubiquitous, and in order to determine whether the latter bug was a host for flagellates of the herpetomonas type a large number of them was dissected, but all with negative results. A number of these living bugs and their nymphs was placed in a jar containing *Lygæi militaris*, the fæces of which were full of flagellates. After three or four days an interesting fact was noticed; some of the bugs were found attached to the bodies of the *Lygæi militaris* and appeared to be sucking their contents; the bugs were dissected, but in no case were flagellates or cysts discovered in their alimentary tracts. Mention may be made here that several feeding and transmission experiments, based on Patton's[1] line of work in India, have been carried out with *Cimex lectularius* and *Ornithodoros savignyi* on cases of kala-azar in the Sudan, and have all given negative results, and one is inclined to consider that these insects cannot be held responsible agents in the transmission of kala-azar in the Sudan.

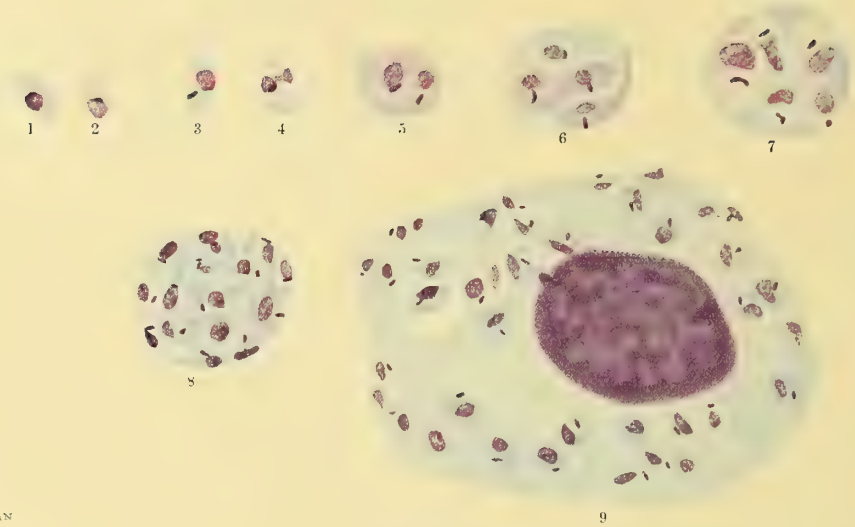
Conclusion

In conclusion, one may say that the herpetomonas which is found to infest the alimentary tract of *Lygæus militaris* in the Sudan is more or less morphologically identical with, and goes through the same life-cycle as, the *Herpetomonas lygæi* described by Patton in India. Animal experiments proved it to be non-pathogenic, and, therefore, it is not at all likely that it is in any way associated with the pathological conditions known as oriental sore and kala-azar.

* Bed-bugs recently sent me from the Lado District, Mongalla Province, proved to belong to this species.—A.B.

[1] Patton, W. S. (1907), "The Development of the Leishman-Donovan parasite in *Cimex rotundatus*." *Scientific Memoirs of the Govt. of India*, No. 31.

PLATE XII



G. Buchanan

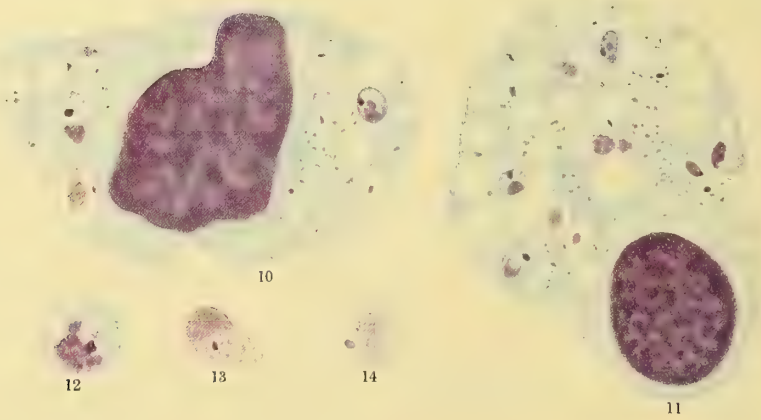
KALA-AZAR

Parasites free and in cells in splenic films before treatment

1–8. Apparent cycle of development to rosette form

9. Heavily infected macrophage

Leishman Stain × 2000 *diam.*



G. Buchanan

KALA-AZAR

Degenerated parasites as seen in splenic films after treatment with "606"

10–11. Note the chromatin granules in the macrophages

12–14. Swollen and degenerated free parasites

Leishman Stain × 2000 *diam.*

Case of Kala-azar treated by "606"

by

The Director

and

Captain R. G. Archibald, M.B., R.A.M.C., attached E.A.

Pathologist and Assistant Bacteriologist

Wellcome Tropical Research Laboratories, Khartoum

As we were desirous of testing the new drug, dioxydiamidoarsenobenzol, or "606," on protozoal infections in the Sudan, and more especially on cases of kala-azar and fowl spirochætosis, a request was sent to Professor Ehrlich asking him if he could kindly spare a supply of his remarkable preparation. He most generously despatched a considerable quantity of the drug and also favoured us with full instructions for its administration, his views as to the type of cases in which it should not be employed, and the precautions which he considered advisable in its use. Its action on fowl spirochætosis is discussed elsewhere (*vide page* 99), but Captain Byam, the S.M.O. of Kassala, having been good enough to send a case of kala-azar, in a Sudanese boy, to Khartoum, expressly for the purpose of treatment with the new preparation, we were able, by the kind permission of Captain A. G. Cummins, R.A.M.C., who had charge of the case in Khartoum, to observe the action of dioxydiamidoarsenobenzol on a disease caused by one of the most resistant of hæmatozoa, and on a fever which is one of the most intractable in the Tropics.

Summary of the case

The following are Captain Byam's notes on the case up to the time the boy left Kassala. They are accompanied by the temperature chart (Fig. 37) shown on *page* 188.

Hassan Said. Age 10 years.

September 6, 1910. Reported sick at Civil Hospital, Kassala, stating that he had been ill with "fever" for the previous three months. Admitted to hospital. On examination, general condition good and patient apparently in no pain. Spleen extended to one hand's breadth below costal margin.

September 13, 1910. Has suffered with daily remittent fever. Placed on quinine.

September 21, 1910. Examination of peripheral blood having shown no malarial or other parasites on several occasions, splenic punctures performed. Leishman-Donovan bodies discovered in large numbers, mostly free.

September 22, 1910. Isolated. Father, mother and two brothers examined and found healthy. Has always lived in Kassala to the west of River Gash.

Tukl and all personal belongings burnt. Treatment with senega (tincture) commenced. Quinine by mouth and injection has had no effect on the fever, though the spleen varies, almost daily, in size. No diarrhœa or profuse sweating.

October 24, 1910. Has recently increased in weight. By sick convoy to Khartoum.

On arrival in Khartoum the patient was admitted to the Khartoum Military Hospital on November 6, 1910. His general condition was fair. The most noticeable sign was a very prominent abdomen, which was tight as a drum. The skin was somewhat dry but showed no evidence of the presence of any petechial spots. The conjunctivæ were

a little pale. Jaundice was absent. The tongue was clean and the teeth were in excellent condition. No abrasions or ulcers were present in the gums or mucous membrane of the mouth. There was no evidence of either a general or a circumscribed œdema being present. Epistaxis and diarrhœa were absent and no tremors were visible.

On palpating the abdomen, enlargement of the spleen and liver could be detected. A careful examination failed to reveal the presence of ascites.

The spleen measured 5 inches in its longest diameter. It was very firm to the touch and had well-defined edges. Its lowest limit was on a level with the umbilicus. The greatest length of the liver in the parasternal line was five inches. The patient's girth measurement, taken at a point 2 inches below the apex of the xiphisternum, was 27 inches.

Neither the spleen nor liver were tender on palpation.

Diagnosis confirmed by spleen puncture

Spleen puncture was performed by one of us (R.G.A.) on November 7, and the smears made were examined and showed the presence of a large number of Leishman bodies, indicating a very heavy infection with these parasites. Not only were there the usual types of Leishman bodies, but there were also seen peculiar rosette-like forms (Plate XII., fig. 8), in which six or more parasites were found clumped together and surrounded by a cell wall. Dividing forms were also present like those described and figured by Visentini[1] (Plate XII., figs. 5–7). Rapid multiplication of the parasites was evidently taking place.

It may be explained here that this spleen puncture was carried out for the purpose of serving as a control experiment prior to the administration of the drug "606."

Three films taken from the peripheral blood showed the presence of two parasites, both of which were phagocyted inside leucocytes.

A differential leucocyte count showed the following percentages :—

Polymorphonuclear leucocytes	38 %
Large mononuclear leucocytes	39·8 %
Large lymphocytes...	9·2 %
Small lymphocytes...	6·8 %
Eosinophiles	1·2 %
Transitionals	4·8 %
Basophiles	·2 %

The patient's red blood corpuscles numbered 3,133,333 per cm. The white corpuscles numbered 5,176 and the blood showed 60 per cent. of hæmoglobin. The patient weighed 3 st. 8 lb.

Injections commenced

On November 8, ·2 gramme of "606," in liquid paraffin, was injected into the gluteus maximus muscle. In the evening the temperature fell two degrees lower than that recorded the previous night. The patient appeared to be fairly comfortable but complained of a little pain. On the following day the report of the patient was favourable, but towards evening his temperature rose, possibly as a result of his getting out of bed and walking about without permission.

On November 10 the patient's condition appeared to be quite good and no ill-effects were apparent as a result of the injection.

[1] Visentini, A. (July, 1910), "Über die Morphologie und den Entwicklungskreis der bei Kranken Kalabriens und Sigiliens beobachteten Leishmania." *Archiv. f. Schiffs und Tropenhygiene, Beiheft* 4.

Examination of three peripheral blood smears revealed the presence of a single Leishman body phagocyted by a large mononuclear. The trophonucleus of the parasite stained faintly while the kinetonucleus appeared as if it was being extruded.

On the 11th the patient had a slight epistaxis, otherwise his condition remained much the same.

Three days after, a swelling appeared in the buttock at the site of inoculation. This was opened and found to contain serum, due possibly to the fact that the drug had been injected into a bursa. Peripheral blood films were taken and a single typical parasite was found ingested by a mononuclear leucocyte. A differential leucocyte count of 500 cells showed :—

Polymorphs	53 %
Mononuclears	13 %
Large lymphocytes...	19·2 %
Small lymphocytes...	10·8 %
Eosinophiles	·6 %
Transitionals	3·4 %

The increase in polymorphonuclear leucocytes was in all probability dependent upon the swelling at the site of the first inoculation.

Second injection

On November 15, ·4 gramme of "606," suspended by Wechselman's method in neutral NaCl solution (physiological), was injected subcutaneously below the scapular region. No ill-effects followed this second inoculation, in fact the patient's condition showed marked improvement. His weight was now 3 st. 13 lb., showing an increase of 5 lb., and the spleen had diminished appreciably in size. On November 20 peripheral blood films were examined, and a single parasite was found inside a mononuclear leucocyte. One megaloblast and two normoblasts were found to be present. A differential leucocyte count showed :—

Polymorphs	38·6 %
Mononuclears	22 %
Large lymphocytes	19·8 %
Small lymphocytes	13·8 %
Eosinophiles	·4 %
Transitionals	4·8 %
Basophiles	·4 %
Myelocytes	·2 %

Judging from this examination there was a change for the worse in the patient's blood. However, in spite of this, the improvement in his physical condition continued, and on November 26 he weighed 4 st. His peripheral blood was again examined

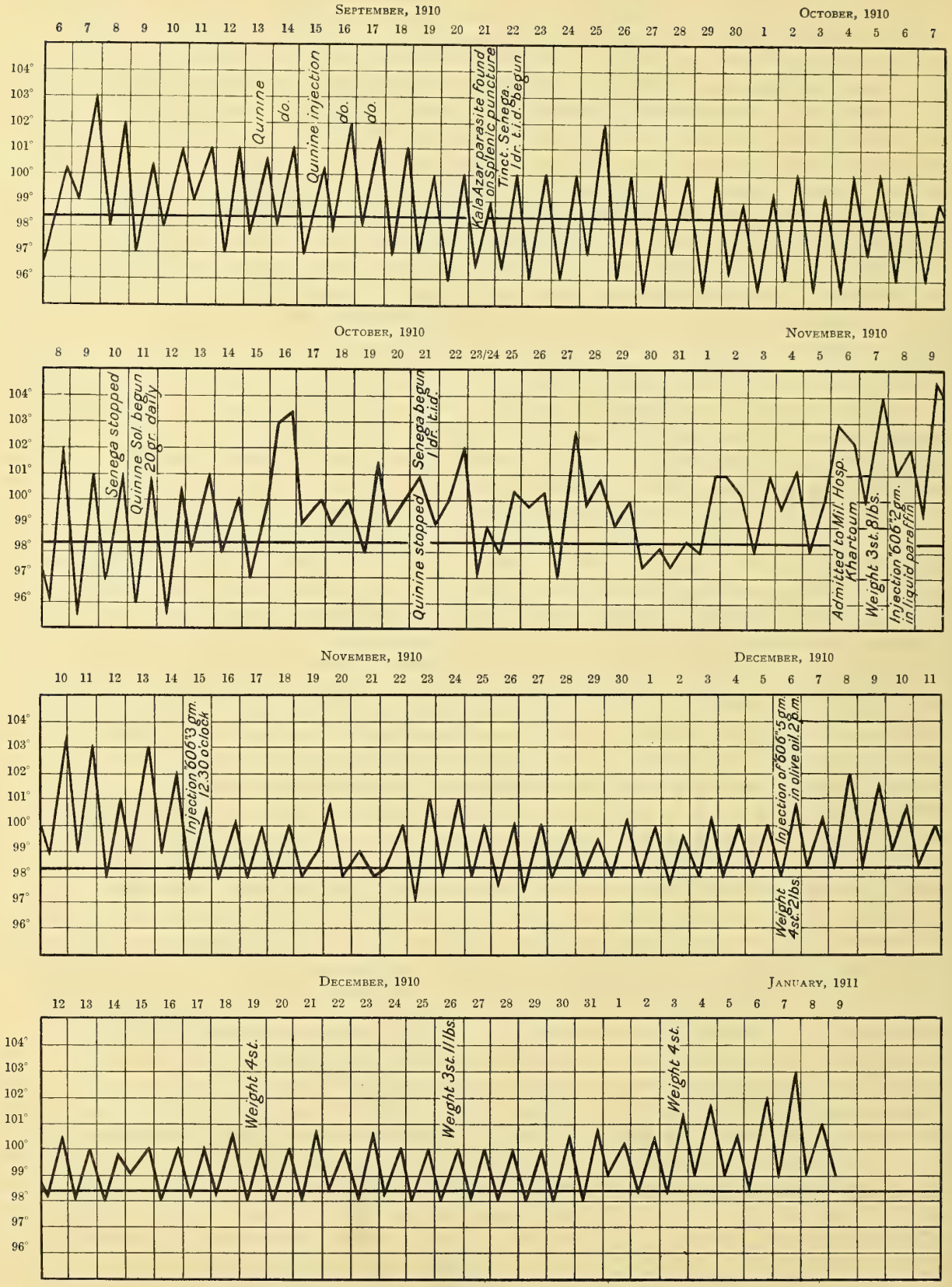


Fig. 37.—Temperature Chart, Case of Kala-azar treated by "606"

for the presence of Leishman parasites but with negative results. One megaloblast was present in the films. A differential leucocyte count showed:—

Polymorphs	37·4 %
Mononuclears	22·2 %
Large lymphocytes	24·6 %
Small lymphocytes	8 %
Eosinophiles	·2 %
Transitionals	7·4 %
Myelocytes	·2 %

Improvement

The patient's health continued to improve, his weight going up to 4 st. 2 lb. The spleen had diminished in size and now measured 3 inches in its greatest length. The liver showed no alteration, still measuring 5 inches in the parasternal line, and the patient's girth at a point two inches below the xiphisternum still remained 27 inches.

It was now considered advisable to carry out another splenic puncture in order to see what effects the drug "606" had produced on the Leishman parasites. Accordingly, a spleen puncture was made and the smears examined.

Effect on parasites

Many unchanged parasites were present. The rosette forms were also seen in fair numbers, but in certain parts of the films, especially along the edges, there were clumps of Leishman bodies which had undergone degenerative changes. In many of them the trophonuclei had disappeared and the kinetonuclei had broken up into granules. Many of the large endothelial cells showed gaps in their cytoplasm. These spaces looked as if they had contained parasites which had vanished from them. Plate XII., figs. 10–14.

On December 6, ·5 gramme of "606," in sterile olive oil, was injected into the buttock.[1] This was not followed by any unpleasant symptoms.

On December 11 another differential count was carried out. The patient's general condition had distinctly improved. The spleen was smaller, the abdomen was quite lax. The count was as follows:—

Polymorphs	37·6 %
Mononuclears	21·8 %
Large lymphocytes	26·4 %
Small lymphocytes	7·2 %
Transitionals	6·6 %
Basophiles	·4 %

No myelocytes, normoblasts or megaloblasts present. One Leishman parasite was found lying free on a red cell. The parasite showed no evidence of degeneration.

December 23.—No further change has occurred except that for a few days there was a slight loss of weight, but at the time of writing the loss has been made good and the patient's condition remains satisfactory.

[1] This form of administration was suggested by Captain W. B. Fry. We note that it has recently been employed by Volk.[2]

[2] Volk R. (1910). *Wien. Med. Klin.* No. 35.

January 7, 1911.—The patient appears to have improved in health. The spleen has diminished considerably in size, and gives the impression of being firmer in consistence. There is no change in the size of the liver. A spleen puncture was carried out and examination of the stained films showed that the parasites had increased considerably in number, but there were evidently degenerative changes occurring in some of them. Many of them had lost their contour and showed vacuolation of the trophonuclei and their cytoplasm. The large rosette forms present were considerably vacuolated and did not appear to be so numerous as formerly. Some of the spleen juice, removed by puncture, was inoculated into the peritoneal cavity of a *Cercopithecus sabæus*.*

Remarks

Results, though not conclusive, are encouraging

The case, not having reached any definite conclusion, little need be said as regards the course of treatment and the effects of the drug. On the whole, the latter seem to have been favourable, but there has been no *therapia sterilisans magna* such as is said to occur in syphilis and at which Ehrlich is aiming. The parasites, however, certainly seem to be somewhat susceptible to the drug, and, though it is difficult to be certain, the last examination would appear to indicate that multiplication forms have been discouraged, although single forms of *Leishmania* are possibly more numerous. These, however, show evidence of degeneration in not a few instances. The case is one of very severe infection, and at first the parasites were reproducing themselves on a large scale. From our experience of similar cases here we are bound to say that we would have expected the boy, if untreated, or treated in the usual manner, to have lost ground rapidly. Hence we are disposed to think that the drug has been of benefit, but one must remember that even bad cases of kala-azar may recover, or at least show temporary improvement. Hence it is well to suspend judgment at present, but it may be advisable to try intravenous injection if it is found possible to persuade the boy's father to remain in Khartoum and agree to an extension of the treatment.[1]

A further trial of the drug would also seem indicated in any other cases of the disease which may come under notice.

We notice that recently Nicolle and Conor[2] have been employing "606" in the treatment of infantile kala-azar both in the child and in infected dogs. They record the case of a dog, heavily infected, in which the drug caused a complete disappearance of the parasites from the spleen and bone-marrow.

Note

Captain Byam, R.A.M.C., S.M.O. Kassala, reports that in April, 1911, the boy was still alive, and, he thought, in rather better health than formerly.

* This monkey never became infected though one which was inoculated in a similar way before the administration of any "606" became heavily infected and eventually died.

[1] This could not be done. The boy was removed to his home in the Kassala Province and the record accordingly remains incomplete.

[2] Nicolle, C., and Conor, A. (December 14, 1910), "Application du '606' traitement du Kala-azar." *Bull. Soc. Path. Exot.*

Two Cases of Non-Ulcerating "Oriental Sore," better termed Leishman Nodules

by

The Director

with Clinical Notes by

Captain D. S. B. Thomson, M.B., B.Ch., B.A.O., D.P.H., R.A.M.C.

Attached Egyptian Army

Introduction

There came recently under the care of one of us (D.B.T.), an Egyptian soldier (Case I.) with a peculiar skin lesion, the character of which is well shown in the accompanying photographs taken by Dr. Beam. The patient was sent to the Wellcome Tropical Research Laboratories, where it was suggested that it would be well to examine the contents of the skin lesions and also to make a histological examination of the growths. This was done by one of us (A.B.) with the result that large numbers of a species of Leishman-Donovan body, presumably *L. tropica*, were found, but while some sections of the growths bore a close resemblance in their pathological histology to true oriental sore, others presented peculiar features. Indeed, both clinically and histologically, the condition was at first suggestive of that rare skin disorder which bears many different names, but is perhaps best described as benign multiple cystic epithelioma or epithelial cystadenoma of the skin. The history showed that we were dealing with a disease of considerable interest, an interest not lessened by the discovery of a second very similar case, which, in all probability, had become infected from the first.

It is proposed in the present paper to discuss the condition generally, to give the clinical histories of the two cases, and to deal with the microscopic findings, while mention will also be made of certain efforts to cultivate the parasite, to carry out animal inoculation and as regards the question of prophylaxis and treatment.

Case I.—Ahmed Hussein (Fig. 40), soldier in No. 4 Company, 16th Battalion, Egyptian Army, aged 20, an Egyptian fellah from the village of Nezli Bedeni in the Mudirieh of Minieh, Upper Egypt. He is a stoutly built man, not very intelligent, in good condition, but not of such fine physique as is usually seen amongst the Egyptian soldiery. He has had five months' service in the Egyptian Army, the last four months of which have been spent in the Sudan.

Admitted to the Military Hospital, Khartoum, on October 9, with soft, pink, keloid-like, raised growths on the face, neck, shoulders, arms, back and inner surface of the thigh.

Family history.—Father, aged about 70, developed *similar growths six years ago*, and at the present time they are more numerous and much larger on his body than on that of his son. Since the growths appeared he has become a lunatic and has lost his hearing. The tumours are said to be in some instances many inches in diameter, and *have never showed any signs of ulceration or breaking down.*

Mother dead, cause unknown.

Brothers: One brother and one step-brother. Neither is affected. The former, aged about 40, would appear to be phthisical.

Two of the patient's immediate relatives affected

Sisters: Six in number and all unaffected save one. Eldest, aged about 35. Youngest, about 12 years. The eldest sister is married and has three girls, all healthy. The second sister has two children, a boy and a girl, both healthy, the third sister has one girl who is healthy, *but the mother has for the past four and a half years suffered from the same skin disease as her brother.* She became infected when living at home, and in her case the tumours are about as numerous as in the brother's. Her husband shows no sign of the disease.

The fourth sister has one girl, who is healthy. The two youngest sisters are unmarried.

A paternal uncle died of phthisis four years ago. An aunt, aged 75, is healthy. Patient's grand-parents are dead, cause of death unknown.

Personal history.—As a child, patient had small-pox. There is no history of any venereal complaint. No history could be obtained as regards the presence of insect parasites in his house or village.

History of present illness.—The growths, which, as will be seen, occur for the most part in groups (Fig. 41), began to appear first of all upon the left side of the neck, two years ago, when he was living in his native village. Then the left side of the face became infected eight months ago, followed by the right shoulder and right upper arm six months ago. Four months ago the neoplasms appeared on the outer surface of the left forearm, and fourteen days ago very small papules presented themselves on the inner surface of the left thigh, about the region of Scarpa's triangle.

In addition, growths, the date of appearance of which is uncertain, are present over the insertion of the left deltoid, and nearly in the centre of the back slightly to the left of the spinal column.

The growths are said by the patient to appear first as small pink circular points raised above the surface of the skin and of about the size of a pin's head. They grow until they reach the size of a pea, when secondary points appear to develop at the periphery of the primary growths, and as a rule separated from these latter by narrow tracts of healthy skin. These secondary tumours develop and become absorbed into the parent growths. As a result, the main tumours increase in size and the whole mass takes on an irregular shape.

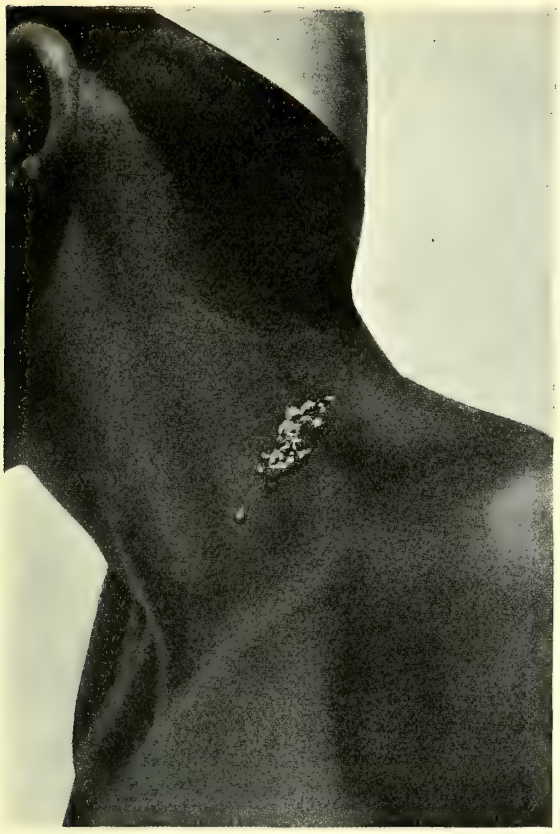
The papules considered "unclean" by the natives

The patient complains that the growths itch intensely when exposed to the sun's rays, and that they pain him when pressed or when they come violently into contact with anything hard.

The people of his village pronounced him "Wāāsh," *i.e.* unclean, and refused to eat with him.

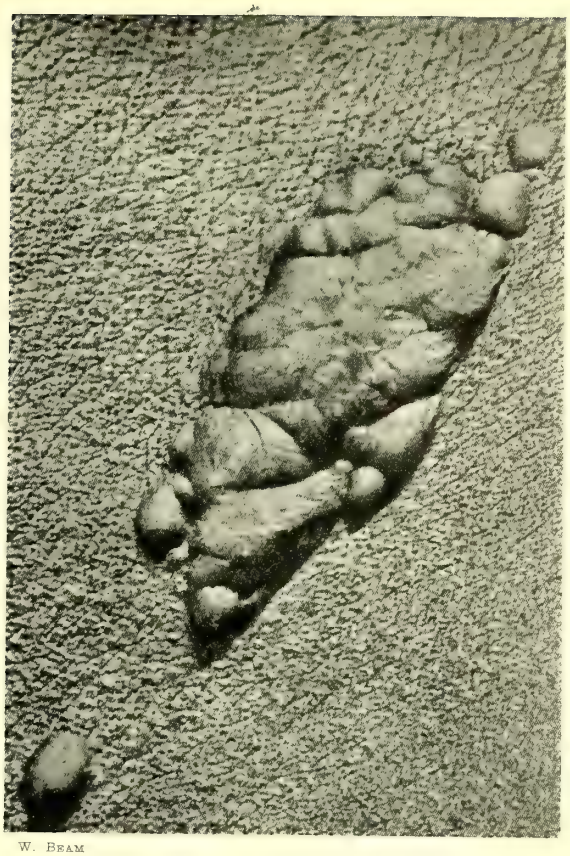
Clinical examination.—General appearance healthy, expression dull and apathetic, no wasting, no sign of any systemic disease, no glandular enlargement. Heart, lungs, liver and spleen normal. Urine: no sugar, no albumen.

The growths in the situations indicated resemble nothing so much as the mountains on a relief map (Fig. 39) looked on from above. There is the main elevation with spurs and ridges projecting from it, these latter representing the secondary growths which have united with the parent lesions. The tumours are of a definite pink colour, contrasting well with the brown pigmented skin, have a shiny aspect, are neither scaly nor ulcerated, and show no signs of breaking down. To the touch they feel smooth, firm, yet soft in consistency, are easily moveable and are not adherent to the deeper tissues (Fig. 38).



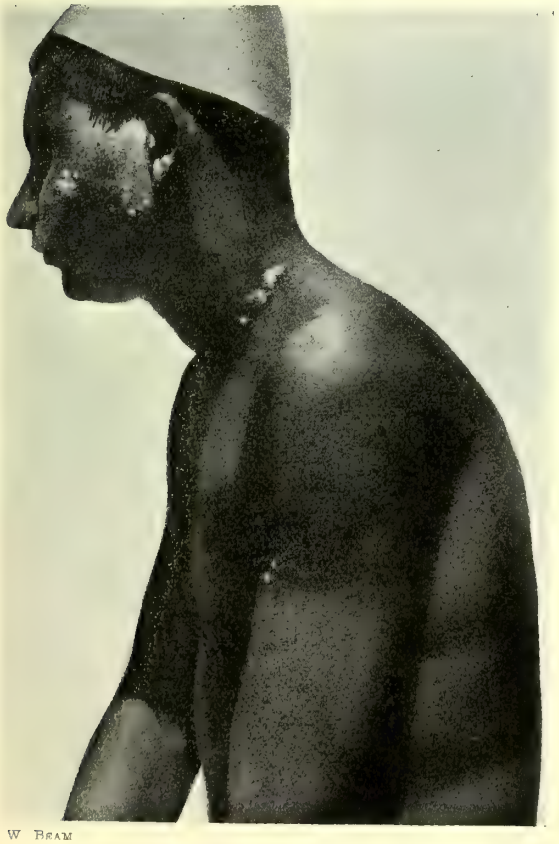
W. Beam

Fig. 38.—Case 1. Neck growth



W. Beam

Fig. 39.—Case 1. Neck growth. Twice natural size



W Beam

Fig. 40.—Case 1. Growths on face, neck and left arm



W. Beam

Fig. 41.—Case 1. Growths on right shoulder and upper arm
Those showing scabs have been punctured

When punctured they either yield blood alone, or blood with serum, or blood and a small quantity of white cheesy sebaceous-like material. After puncture, the site of injury heals readily, and a small scale or scab forms. This is well seen in Fig. 41. On section to the naked eye the growths appear fibrous. The following are the measurements of the growths :—

Rapid healing after puncture

On the face	20 mm. by 18 mm.
On the neck...	35 mm. by 15 mm.
Over insertion of left deltoid ...	5 mm. by 5 mm.
On outer surface left forearm ...	not measured—excised
On right shoulder	20 mm. by 14 mm.
On right upper arm	29 mm. by 18 mm.
On left thigh	$2\frac{1}{2}$ mm. by $2\frac{1}{2}$ mm.
On back	$2\frac{1}{2}$ mm. by $2\frac{1}{2}$ mm.

Blood examination.—An examination of blood taken from the finger, made on October 10, showed 6,050,000 red blood corpuscles and 5,600 leucocytes. *Leucopenia therefore is present.* The hæmoglobin was not determined—the blood was of a good colour. Its coagulability was markedly increased. A differential leucocyte count was made with the following result :—

Eosinophiles	11·6 per cent.
Polymorphs...	57·4 ,,
Mononuclears	4·2 ,,
Large lymphocytes	8·6 ,,
Small lymphocytes	16·2 ,,
Transitionals	2·0 ,,

Characteristic differences between the blood from the papules and that from the general circulation

With the exception, therefore, of a marked eosinophilia, the result probably of infection with metazoan parasites, so common in these fellaheen, the count was normal. No protozoal parasites of any kind were found.

Very different was the differential count in blood taken at the same time from the growth on the neck. It gave :—

Eosinophiles	2·2 per cent.
Polymorphs...	26·8 ,,
Mononuclears	23·6 ,,
Large lymphocytes	40·8 ,,
Small lymphocytes...	3·2 ,,
Transitionals	3·2 ,,
Basophiles	0·2 ,,

There were many free nuclei of disintegrated mononuclears. Here, then, we find a marked increase of the larger mononuclear cells at the expense of the polymorphs.

The blood was taken by means of a fine capillary pipette thrust through a small needle puncture into the depth of the growth. This slight operation was not unattended with pain, the patient wincing and complaining.

Before going further, it is interesting to compare these results with those obtained by Cardamatis[1] in several cases of oriental sore in Greece. In three cases he took blood from the finger and from the congestive zone at the periphery of the lesions. His counts are not at all unlike those recorded on *page* 194, and he found in the blood from the congestive zone the same marked increase of large mononuclear elements as we record in the case under discussion. Indeed, as regards this third case, the counts are almost identical. As mentioned, blood taken from the growths by puncture and made into films in the usual way, when fixed and stained by the Leishman method, showed parasites belonging to the genus *Leishmania*. It was thought advisable to examine the contents of nearly every one of the growths. It will be best to consider these briefly in detail.

Presence of *Leishmania* in the growths

1. *Face growth.*—Parasites numerous, both free and in the mononuclears. None seen in the polymorphs. Many of the mononuclears are crowded with them, thirty being no uncommon number in one cell (Fig. 42). Some of these mononuclear cells are very large with much extra-nuclear protoplasm, and are evidently the large endothelial cells commonly found infected in cases of kala-azar and oriental sore. The free parasites occur singly, in pairs and in the larger groups which have been so often described by various observers. As many as 12 lying in close apposition were counted in one clump. The blepharoplasts are both rod-shaped and spherical. Single, somewhat large forms with curved blepharoplasts noted. In addition, there are small coccoid bodies which may be free nuclei or special parasitic forms, and there are also somewhat pear-shaped or wedge-shaped cells staining a light rose-pink, and exhibiting each a small spherical nucleus, but no blepharoplast.



G. Buchanan

Fig. 42.—Parasites free and grouped together in the cytoplasm of a large mononucleated cell.

2. *Neck growth.*—The parasites are not so numerous as in 1. They show vacuoles better, and occur both free and in the mononuclears. Curved blepharoplasts were again noted in some of them. Blood and white cheesy matter, mixed, were also obtained from this growth, kept in a sealed, sterile capillary tube for six hours at room temperature (about 35° C.), and then smeared and stained. Parasites were found free and in mononuclears, and groups of what seemed to be large cocci were observed, as was a number of pale blue homogeneous structureless masses with regular outlines, probably formed from the matrix of ruptured host cells.

Coccoid bodies

3. *Left upper arm growth.*—Parasites fairly numerous. Coccoid bodies and pink pyriform and wedge-shaped cells present. Small clumps comparatively common. A large

[1] Cardamatis, J. P. (May 12, 1909), "Leishmaniosis en Grèce (Bouton d'Orient)." *Bull. Soc. Path. Exot. Paris.*

"Large blue cells" resembling certain appearances in Kala-azar films

cell noted, which stains a pale blue and contains a faint indication of what may be a large nucleus, although the latter stains more faintly than the cell protoplasm. Similar large blue cells have been seen in splenic smears from cases of kala-azar.

4. *Left forearm growth* (subsequently excised).—Serum and a little blood were obtained. There were many parasites in the mononuclears. Cells like degenerated leucocytes present.

5. *Right shoulder growth.*—Large numbers of parasites, both free and in the mononuclears. Free clumps well seen. Degenerated white cells noted.

6. *Right upper arm growth.*—Parasites very numerous, and are more spherical in shape than in the other films. Mononuclears crowded with them. Vacuoles well marked. Rod-shaped, curved and spherical blepharoplasts. One form noted with two nuclei, a well-marked vacuole and a single curved blepharoplast. This possibly represents a dividing parasite. Some very curious masses observed, either the result of fusion or of division. A small accessory growth situated close to the main tumour also showed the parasites.

7. *Growth on back.*—A film made from the contents of this small growth showed no parasites.

8. *Thigh growths.*—From these tiny tumours only white cheesy sebaceous-like matter was obtained. Films were made of it, and coccal forms of two kinds were found—one resembling those seen in the smears of similar material from the neck growth and taking on a deeper stain than the other, which consisted of small cocci of the usual type, occurring largely as diplococci.

Morphology of parasites.—For the most part the parasites conform to the typical *L. tropica,* and do not require special and detailed description. Practically all the forms which have been described in oriental sore are present, and careful examination has not detected differences, either in structure or arrangement.

One need only mention the curved blepharoplasts, which struck us as peculiar, and the bodies staining a rose-pink. Examination of sections stained with eosin and hæmatoxylin showed these latter to be plasma cells.

Peculiar "large cocci" found in the shoulder growth

The special form of large cocci found in the growths containing no Leishmania and in the cheesy matter from the neck tumour also claims attention. They are four to six times the size of the small cocci present, and tend to stain very feebly at their centres. Indeed, some of them present centrally-placed unstained areas. They may occur in clumps or in pairs, and then may resemble in some measure huge gonococci. It is not likely that they are concerned with the Leishmania infection save in the way of symbiosis. Carter[1] has pointed out that *L. tropica* grows best in culture when associated with masses of cocci and bacteria.

Although the suggestion, as regards the origin of the blue homogeneous masses, may be correct, it is curious that many of them are very regular in outline, spherical or oval, and resembling, as one may say for want of a better term, "washed out" lymphocytes whose nuclei have vanished.

The white cheesy matter is no doubt sebaceous in origin.

Histo-pathology of growths.—The first growth excised was a small one, accessory to that on the left forearm, subsequently removed.

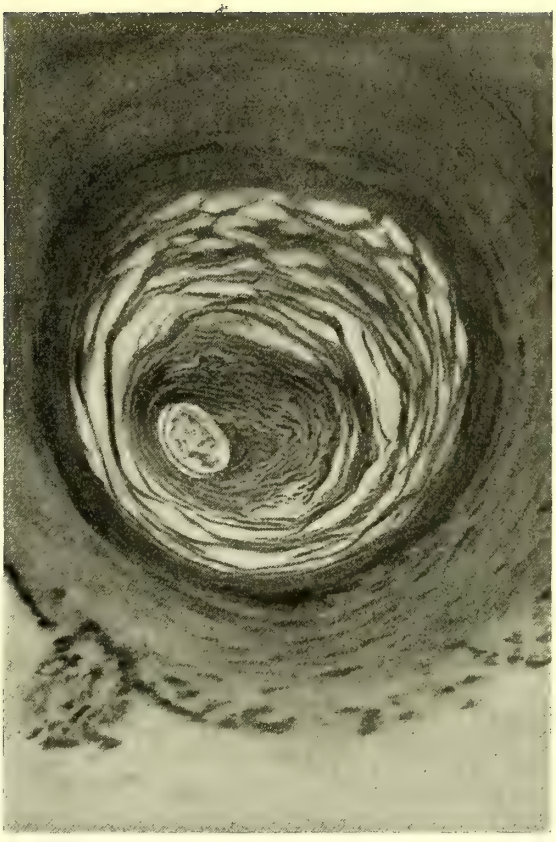
The tissue was fixed in formol alcohol, hardened in alcohol, cleared in xylol, imbedded in paraffin and, after the usual treatment, stained in a variety of ways. The earlier sections were slightly thick, and at first glance exhibited all the appearance of a squamous

[1] Carter, Captain R. M. (September 11, 1909), "Oriental Sore of Northern India. A Protozoal Infection." *British Medical Journal.*



W. Beam

FIG. 43.—Section of small growth first removed, showing cell nests and cellular invasion of the rete Malpighi



W. Beam

FIG. 44.—Cell nest showing pigment in central inclusion



W. Beam

FIG. 45.—Section of second growth excised, showing downgrowths of rete. The cellular infected layer is well seen



W. Beam

FIG. 46.—Section of small growths first removed, showing proliferation of the rete Malpighi

"Cell nests"

carcinoma of the skin. The most prominent feature is the presence of numerous typical cell nests (Fig. 43) which, according to Lazarus-Barlow[1] may, for all practical purposes, be taken as diagnostic of that condition. Leaving the clinical history out of account, however, it was soon evident that we were not dealing with a malignant growth. There is an increase in the rete malpighi, long branching columns of which stretch down into the underlying tissues (Figs. 45 and 46), but there is no actual invasive process, and there are no isolated masses of prickle cells. In these respects, the condition resembled a papilloma. The cell nests, however, are most typical. At the centre of one of the nests (Fig. 44) there is a clear glistening oval body about 28μ by 18μ, containing numerous grains of what is undoubtedly pigment. It is evident that there has been a hyperchondrification of the stratum corneum, as the body stains in the same way as the horny layer, giving rise to this curious appearance, an appearance not unlike a large molluscum body but clearer, more glistening and, as mentioned, studded throughout with pigment. It is known that in the negro's skin pigment granules may be traced from the stratum granulosum to the outermost layers of the stratum corneum. The same is no doubt true, though to a less extent, of the Egyptian. The cells of the rete show marked perinuclear vacuolation, while the papillary layer of the skin is hypertrophied, or, at least, appears to be so, invading and even cutting off and disintegrating portions of the rete, while itself invaded by infiltrating cells. There are islands of cellular tissue studded about in the mass of the prickle cells (Fig. 43). In

Histo-pathology of the growths

these earlier sections the sub-epidermic tissue presents the appearance of a cellular connective tissue undergoing inflammation and some proliferation, and does not apparently conform to the descriptions, in English text-books and papers, of sub-epidermic tissue in oriental sore, albeit examination with high powers of the microscope showed that the cytoplasm of numerous large cells with vesicular nuclei was full of Leishman bodies. These showed up best in sections stained by Van Gieson's method or by hæmatin. Free parasites were also visible in the dilated capillaries, in mononuclear leucocytes lying amongst the cells, no doubt as a result of diapedesis, and between the cells themselves, presumably in the sero-fibrinous effusion. Some of the smaller vessels in this tissue showed distinct endarteritis.

At this stage one may quote the description given by Fordyce[2] of the histo-pathology of multiple benign cystic epithelioma. He says that under the microscope the tumours are seen to be "made up of irregularly rounded, oval and elongated masses and tracts of epithelial cells, corresponding to those in the lowermost layer of the epidermis, and in the external root-sheath of the hair-follicle. The epithelial masses may be distinct or made up of inter-communicating bands and tracts, in some places resembling coil-ducts. *Cell 'nests' are met with as in malignant epithelioma, enclosing horny, granular and colloid tissue.* Colloid degeneration of individual cells is also encountered in the cell masses. The connective tissue about the cell collections is somewhat condensed, but is not the seat of any inflammatory process."

It must be confessed that for the most part this description applied very well to the earlier sections studied, and it was found that Allan Jamieson,[3] quoting various authorities, speaks of epithelial cystadenoma as a species of acanthoma, while the description he gives does not differ markedly from that stated above.

Recent literature referring to allied conditions

It will perhaps be interesting also to consider some of the latest utterances in English or American text-books on the histo-pathology of true oriental sore.

[1] Lazarus-Barlow, W. S. (1903), *The Elements of Pathological Anatomy and Histology.*
[2] Fordyce, — (November, 1894), *Journal of Cutaneous and Genito-Urinary Diseases.*
[3] Jamieson, W. Allan (1901), *Skin Diseases in Gibson's Text Book of Medicine.*

Manson[1] says little—"Section of the papule displays an infiltration of the derma by a mass of small round granulation cells. These lie between the elements of the tissues, particularly about blood-vessels, lymphatics and sweat-glands; towards the centre of the lesion they completely replace the normal structures."

Wright,[2] in his original paper, states that "the lesion consists essentially of a very extensive infiltration of the corium and papillæ by cells, accompanied by atrophy and disappearance of the epidermis of the part. The infiltrating cells are plasma cells, various kinds of lymphoid cells, and large cells with single vesicular nuclei and a relatively large amount of cytoplasm, in which are large numbers of the micro-organisms. These large cells, over extensive areas, are very numerous, and constitute the principal part of the infiltration. They are regarded as proliferated endothelial cells."

Brooke[3] mentions the researches of Carter, Reihl, Leloir, Unna and Kuhn, "who all found it to be a chronic sero-fibrinous inflammation. There is a round-celled infiltration of the skin and subcutaneous tissue. In the centre of the nodules this infiltration is so dense that the tissue elements are entirely disintegrated. The lymphatic vessels are dilated and there is much œdema. Necrosis proceeds in the centre of the infiltration tissue. There is cornification of the hair root-sheaths, preventing a subsequent new growth of hair at the spot. There is endothelial proliferation of the blood-vessels, often leading to their entire obliteration."

"A chronic Sero-fibrinous inflammation"

Christophers and Stephens,[4] in their latest edition, do not deal with the subject, nor does Daniels.[5] The English translation of Scheube's[6] work has a passage much like that in Brooke's book:—

"There is an infiltration of the skin and subcutaneous tissue, with small round oval cells, multi-granulated and giant cells, and also a few leucocytes. In the centre of the nodules, over which the epithelium is attenuated, the infiltration is so dense that the tissue elements are entirely disintegrated, while towards the periphery the cells form small centres, mostly situated in the vicinity of blood-vessels and lymphatics and sweat glands. The lymphatic vessels and spaces are uncommonly wide, and there is œdema. In the centre of the infiltrated tissue, small necrotic particles are enclosed, and this, when the sections are stained, exhibits large fibrinous contents; the tissue otherwise also contains much fibrin (Unna). Those hairs that still exist are, according to Kuhn, partly broader, partly narrower, occasionally ravelled, and they always exhibit a granular appearance. The root-sheaths are sometimes dilated. Here and there the inner root-sheaths, and to a great extent the outer root-sheaths, are also transformed into a shiny mass, probably cornified. In the connective tissue around the hair follicles, cavities are found filled with shiny flaky masses which, according to the way they are cut on section, appear of a round or oval form. In consequence of the cornification of the root-sheaths no new growth of hair is possible. The blood-vessels exhibit endothelial proliferation, which may lead to their entire obliteration."

"Endothelial proliferation" in blood-vessels

Firth,[7] in Allbutt's *System of Medicine*, has a lengthy description as follows:—

"The histology of these sores has been thoroughly worked out; and, if sections be made of the initial papule before ulceration, no difficulty is experienced in demonstrating

[1] Manson, Sir P. (1907), *Tropical Diseases* (4th Ed.).
[2] Wright, J. H. (December, 1903), "Protozoa in a case of Tropical Ulcer." *Journal of Medical Research.*
[3] Brooke, G. E. (1908), *Tropical Medicine, Hygiene and Parasitology.*
[4] Stephens, J. W. W., and Christophers, S. R. (1908), *The Practical Study of Malaria, etc.*, (3rd Ed.).
[5] Daniels, C. W. (1907), *Laboratory Studies in Tropical Medicine* (2nd Ed.).
[6] Scheube, B. (1903), *The Diseases of Warm Countries.* Edited by J. Cantlie.
[7] Firth, R. H. (1907), In Allbutt's *System of Medicine*, Vol. II., Part II.

that the whole thickness of the skin and subjacent tissue is infiltrated with lymphoid and epithelioid (mesoblastic) cells, accompanied by more or less complete disintegration of the normal tissue-elements thereby. In the centre of the papule the infiltration by young round cells is so complete that little can be seen of the sweat-glands. Towards the edges of the diseased area, the new cells occur in isolated clusters or groups, chiefly round blood-vessels or lymphatics. The infiltration does not seem primarily to involve either the hair-follicles or the sebaceous glands. The individual cells of this infiltration vary from 7μ to 9μ; their nuclei from 5μ to 6μ; the nuclei are large, generally single, but in parts multiple. The anatomical structure of the papule and surrounding skin indicates that oriental sore is of the type of a granuloma; in fact the most elementary microscopical examination of the lesions shows that it is a reaction of the skin against some virus of low virulence, which has produced granulomatous changes in the corium beneath and round the ulcer. So chronic are the changes which are sometimes met with, that a close resemblance to tuberculosis may be occasioned. It is important to bear this in mind, because it has several times been suggested that certain of these lesions are tuberculous. Doubtless, syphilitic and tuberculous ulcers have, from time to time, been placed in this group, but that there is an entity to which the term oriental sore is applicable, which is due to some virus different from syphilis and independent of tubercle bacilli, seems certain."

"A reaction of the skin against some virus of low virulence"

Jackson,[1] on the other hand, states that the pathological anatomy of tropical ulcer needs to be cleared up by further study. None of these accounts, it will be seen, mentions the presence of cell nests, or even the increase in the rete Malpighi, which was so marked a feature in the sections of the small growth first studied. We were inclined to ask if this tumour was taking on malignant features, if it was a true oriental sore in the papular stage, or if these descriptions referred only to the ulcerative stage of the lesion. Manson's brief statement, however, distinctly mentions section of the *papule*.

Another and larger growth of four months' duration, that on the outer surface of the left forearm, was excised and examined. After excision, blood was taken from the cut surface, but no parasites were found in it, nor were they present in blood-stained serum, obtained by thrusting a capillary pipette through this surface and some distance into the tumour mass. This is a point of some interest.

No parasites in blood from cut surface

Thin sections were made of the growth and stained by Leishman's method, with Heidenhain's iron hæmatoxylin, with hæmatin, Van Gieson's stain, eosin and methylene blue, hæmatoxylin and thionin blue, and, following Nattan-Larrier and Bussière,[2] with carbol-thionin.

The appearances presented by these sections were much more like the description quoted. There was not nearly so great a proliferation of the rete Malpighi, though here also, in some parts, a tendency to down-growth and to the formation of cell nests was noted. A study of the sections stained with eosin and methylene blue, however, showed that the cells present in the sub-epidermic layer answered very closely to those mentioned by Wright. Indeed all the classical signs were to be observed, and there could be little doubt that the condition was identical with that found by many observers. In these sections there were not, as a whole, nearly so many parasites as in those of the small growth first examined, and they seemed to be confined to the endothelial cells and the mononuclears. In certain areas, however, sections stained with carbol-thionin and carefully differentiated with spirit, showed a heavy infection of the cells.

[1] Jackson, T. W. (1907), *Tropical Medicine.*

[2] Nattan-Larrier, L. and Bussière, A. (January 13, 1909), "Répartition des Leishmania dans le bouton d'Orient." *Bull. Soc. Path. Exot. Paris.*

At certain points the infiltration of the epidermic layers by the round-celled growth was evident, but at most points there still persisted a considerable layer of the rete.

We could find no evidence of actual necrosis, nor were giant cells visible. It was not until we consulted the recent French work on tropical medicine by Jeanselme and Rist that we found a description of anything like the condition seen in the sections of the first small growth observed. There, however, we came across an excellent account of the histo-pathology of "Bouton d'Orient." These authors state that the dominant change consists in a thickening of the rete Malpighi (hyperacanthos and an incomplete keratinisation of the corneal layer [parakératose]). The prickle cells are separated one from the other by an interstitial œdema, and perinuclear vacuolation occurs. They go on to describe the state of the sub-epidermic tissue in much the same way as other writers, but lay stress on the foci of necrosis and the presence of giant cells. Their diagrams, both of skin sections and of the periphery and centre of a typical inflammatory nodule, are very instructive, and, save for the absence of cell nests and the presence of the giant cells, might apply to our sections of the small growth first removed and studied.

It is then, we think, evident that the skin disease under consideration is an example of what is called oriental sore, but it appears to be a type not prone to ulceration.[1] In this case some of the growths have been present for two years, and, even when punctured, tend to scab and heal immediately.[2] In the father's case the tumours have persisted unchanged, save as regards size, for six years, in the sister's case for four and a half years. The patient has been very closely questioned on these points and is very definite in his replies.

The condition is probably identical with that described by other observers, but of a type not prone to ulceration

Has such a condition been previously reported? With one exception we can find no record of such being the case. Some writers speak of the papules persisting for a long period (Cardamatis[3] mentions one of two and a half months) and others of chronic papules which abort, but in no instance is any indication given that growths may attain the size they have done in this case without showing any sign of breaking down.[4]

The exception mentioned is given by Cambillet,[5] who recently described the case of a small native boy in Algiers, who presented, on the right cheek, a tumour, which, to judge from the photograph given, must be almost identical with the face growth in our case. It commenced as a small papule, increased in size until, at the time the paper was written, it measured three centimetres in diameter, and showed no sign of ulceration or discharge. It had persisted for a year in this state. On puncture it yielded blood and "*de petits grumeaux blancs*," and in smears *Leishmania tropica* was found. He concluded that the case was one of "Bouton d'Orient," and certainly this term is much more applicable to his case and ours than that of oriental sore. The latter, in any case, is a misnomer, as instances have been described from Bahia by Juliano Moreira, and recently from Bauru in Brazil, by Lindenberg,[6] who found Leishman bodies present. His work has been confirmed by Carini and Paranhos.[7] We think cases of this kind

[1] Is it not possible that the result depends upon the reaction of the tissue to the virus? If this be strong the epidermic layers thicken; if weak they are destroyed. It may be so, or special forms of Leishmania may produce their own specific results; or again, symbiosis, with cocci or bacteria, may play a part.

[2] A portion only of the small growth showing the cell nests was excised. The wound healed rapidly, and now the nodule presents much the same aspect as it did before part of it was removed.

[3] Cardamatis, J. P. *loc cit* and (July 21, 1909), "Observations microscopiques sur un bouton d'Orient non ulcéré." *Bull. Soc. Path. Exot. Paris.*

[4] See, however, note at end of this paper.

[5] Cambillet, — (July 21, 1909), "Un cas de bouton d'Orient à Flatters (Alger)." *Ibid.*

[6] Lindenberg, A. (May 12, 1909), "L'ulcère de Bauru ou le bouton d'Orient au Brésil." *Ibid.*

[7] Carini and Paranhos, U. (May 12, 1909), "Identification de l'Ulcère de Bauru avec le bouton d'Orient." *Ibid.*

Suggested title "Leishman Nodules"

might, with advantage, be termed Leishman nodules, as the word nodule sufficiently describes the growth, and practically all are agreed that *L. tropica* plays a part, and probably the chief part, in their etiology. It is true that some are apparently anxious to abolish the term *Leishmania* as applied to the parasite altogether, and substitute for it *Crithidium* or *Herpetomonas*, but the word is now so familiar, and in such general use that it might, with advantage, be retained and applied in its modified form as above indicated. Apart from what has been said there is other evidence pointing to this case being one of Leishman nodules.

1. The condition is known to occur in Egypt.

2. Exposed parts of the skin are chiefly affected, the Egyptian fellah being accustomed to work stripped to the waist.

3. The coagulability of the blood is increased; markedly so.

Carter,[1] however, points out that in oriental sore more than one person of a family or household is rarely if ever attacked, while, as already mentioned, our second case, about to be described, appears, undoubtedly, to have derived his infection from the first, two of whose family also suffered from the disease. Gros,[2] who has published an account of four cases on the Algerian littoral, is of Carter's opinion. In no instance was infection transmitted from his cases to those in contact with them. How close this contact is likely to have been, anyone conversant with Eastern customs and usages knows. Gros concludes that "le bouton d'Orient parait donc peu contagieux."

The account of our second case is therefore likely to be all the more interesting.

Case II.—Yani Suleiman; aged 21. Soldier in No. 4 Company, 16th Battalion, Egyptian Army, an Egyptian fellah from the village of Ben Abied, in the Mudirieh of Minieh, Upper Egypt. He is a sturdy son of the soil, well nourished and more intelligent than his compatriot. He enlisted on the same day as did Case I., and for the last five months the two men have been eating together, and sleeping side by side, while they are in the same section of the same company every parade. Patient denies having ever worn the clothes or boots of Case I.

Admitted October 30, with growths, very like those in Case I., situated on his left thigh, right knee and left foot. On admission, he was noticed also to have a small abscess below the outer canthus of his left eye. This contained pus and was evacuated. It may be said at once that no *Leishmania* were found in the pus.

Family history.—Unimportant. Nothing of interest.

Personal history.—Patient states that as a small child he was possessed of an evil spirit for a year, but otherwise has always been healthy. No venereal history obtainable and no signs of venereal disease.

History of present illness.—The first growth on the anterior surface of the left thigh appeared four months ago, *i.e.* one month after he came into contact with Case I.

The comrade of the first case is infected

A second lesser growth, now absorbed into the first, appeared about the same time as did a third growth, accessory to the fused first and second. A fourth, on the inner surface of the right knee, is three months old, and so is a fifth on the dorsum of his left foot about half an inch from the bases of his first and second toes. The tumours itch and are painful on pressure.

Clinical examination showed the patient to be perfectly healthy, with the exception of the skin lesions. These are of the same type as in Case I. When punctured they yield

[1] Carter, Captain R. M., *loc. cit.*

[2] Gros, H. (June 9, 1909), "L'ulcère à Leishmania (bouton d'Orient) sur le littoral algérien." *Bull. Soc. Path. Exot. Paris.*

blood only, and films of this blood show in every instance *L. tropica*. Those, however, are not nearly so numerous as in Case I. The greatest number was found in the knee growth. They occur both free and in the mononuclears, and present no special features. A good many of them appear to be smaller than those in Case I. The tumours show no tendency to ulceration, and quickly heal under a scab after being punctured. Blood from the skin covering the growths shows no parasites.

The measurements of the growths are as follows:—

On the thigh (1 and 2 combined)	17 mm. by 12 mm.
On the thigh accessory	7 mm. by 7 mm.
On the knee	10 mm. by 10 mm.
On the feet	5 mm. by 5 mm.

Blood examination.—The blood coagulates very rapidly. A blood count made by Major Ensor on November 2, showed 4,700,000 red blood corpuscles, and 16,000 leucocytes. It will be remembered that the patient at this time had a boil on his face, probably the cause of the leucocytosis. After a week the leucocyte count dropped to 12,000, although the patient was being treated by Major Ensor[1] with tincture of senega, according to his method in kala-azar cases. As he was anxious to test the effect of the drug the case was handed over to him and has not been studied so fully by us as was Case I. It is chiefly of interest in that it is an example of the transmissibility of the disease and, apparently, of the same type of the disease. What was the agent of transmission? Was it a bed-bug? This is perhaps the most likely insect; for bugs, *C. lectularius*, are a common pest in barracks tenanted by Egyptian troops. Next, most likely, is a *Phlebotomus*, said by Pressat,[2] to be blamed by the fellaheen in Egypt, and regarded by Sergent and others as a possible vector.

The question of mode of transmission

This and other questions we would leave to a later paper, and conclude by thanking Major Ensor, S.M.O. Khartoum, for help in connection with the cases, and Dr. Beam for the trouble he took to secure good photographs of these interesting skin lesions.

NOTE.—Since this paper was written a second article by Carter[3] has appeared, in which he draws attention to the various types of oriental sore which exist in India, mentions a non-ulcerating form, apparently not identical with that we have described, and like us, takes exception to the term oriental sore, though without putting forward any substitute. He also refers to the possibility of there being different varieties of *Leishmania*, an observation with which we wholly agree, and in the light of which we hope to study the parasites found in the skin lesions described.

ADDITIONAL NOTES BY THE DIRECTOR

The above is the account of these interesting cases as it appeared in the *Journal of the Royal Army Medical Corps* and the *Transactions of the Society of Tropical Medicine and*

[1] Ensor, Major Howard (December, 1909), "The Treatment of Kala-azar by the use of Senega." *Journal of the Royal Army Medical Corps.*

[2] Pressat (1905), *Paludisme et les Moustiques.* Paris.

[3] Carter, Captain R. M. (November 6, 1909), "A Note on Oriental Sore." *British Medical Journal.*

Hygiene. Before it was completed, Captain Thomson had unfortunately to leave for the Blue Nile Province. I was practically alone in the laboratories at the time and, as has occurred only too frequently in the past, found myself unable to cope properly with all the research and routine work. This is probably the reason why my attempts to cultivate the parasite failed, though it is true I wasted a great deal of time in preparing and using the somewhat elaborate medium and following the technique devised by Carter.[1] At that time I had not seen Row's[2] work, or Nicolle's[3] paper in which the agar-sea-salt medium is described. I was only able to examine the cases at intervals, and it so happened that, as a result of painting with tincture of iodine, and perhaps also on account of the puncturings to which they were subjected, the nodules gradually shrivelled up and disappeared, the parasites wholly vanishing from their contents. Hence, by the time I had a little more leisure my material was lost and there has been no opportunity of obtaining more, and although Captain Archibald records a case of cutaneous Leishmaniosis, it was not of the nodular type but answered to the descriptions of skin lesions given by Day and Ferguson,[4] being more allied to the warty forms mentioned by Ferguson and Richards[5] as occurring in Egypt.

Treatment

It is perhaps scarcely worth while giving the progress of the cases in detail. It was noted from time to time, and the effectiveness of the iodine treatment has been mentioned. This was different from the experience of Ferguson and Richards who found that ionisation of their "flat forms," which they think are the same as those here described, only caused a temporary and very slow improvement. Possibly, however, a good deal depends on the size of the lesion. They recommend excision followed by skin-grafting, and certainly this seems the best method of treatment as under the iodine regime our two cases were not well and fit for duty until the middle of January, 1910. Moreover, somewhat unsightly scars were left where the nodules had been. The senega treatment did not seem to have any effect.

Animal inoculations.—I was only able to carry out one experiment, a skin inoculation by scarification in the flank of a guinea-pig. The result was negative, but thanks to the recent work of Nicolle and Manceaux[6] we now know that, under certain conditions, oriental sore can be communicated to the smaller monkeys and to the dog when culture forms are employed.

The goat, cat, sheep, white rat, ass and horse are not susceptible. The guinea-pig is only mentioned indirectly, but it would appear that it can be infected if the inoculation is made in the thickness or the neighbourhood of the skin. Failure, therefore, in the case

Experimental work

mentioned, was probably due to the fact that the material employed was taken direct from the lesion and not from cultures of the parasite. In any case, however, no information of any value can be obtained from a solitary negative experiment.

Mention has been made of the failure of attempts at cultivation on Carter's medium. These also failed in citrate solution, and on ordinary blood agar.

[1] Carter, Captain R. M. (September 11, 1909), "Oriental Sore of Northern India. A Protozoal Infection." *British Medical Journal.*

[2] Row, R. (1909), "Development of Flagellated Organisms from the Parasite of 'Oriental Sore.'" *Transactions Bombay Medical Congress.*

[3] Nicolle, C. (April, 1908), "Recherches sur le Kala-azar, Isolement et Culture des Corps de Leishman." *Arch. de l'Inst. Past. de Tunis.*

[4] Day, H. B., and Ferguson, A. R. (November 1, 1909), "An account of a form of Splenomegaly with Hepatic Cirrhosis, Endemic in Egypt." *Annals of Tropical Medicine and Parasitology.* Liverpool.

[5] Ferguson, A. R., and Richards, Owen (July 25, 1910), "Parasitic Granuloma." *Annals of Tropical Medicine and Parasitology.* Liverpool.

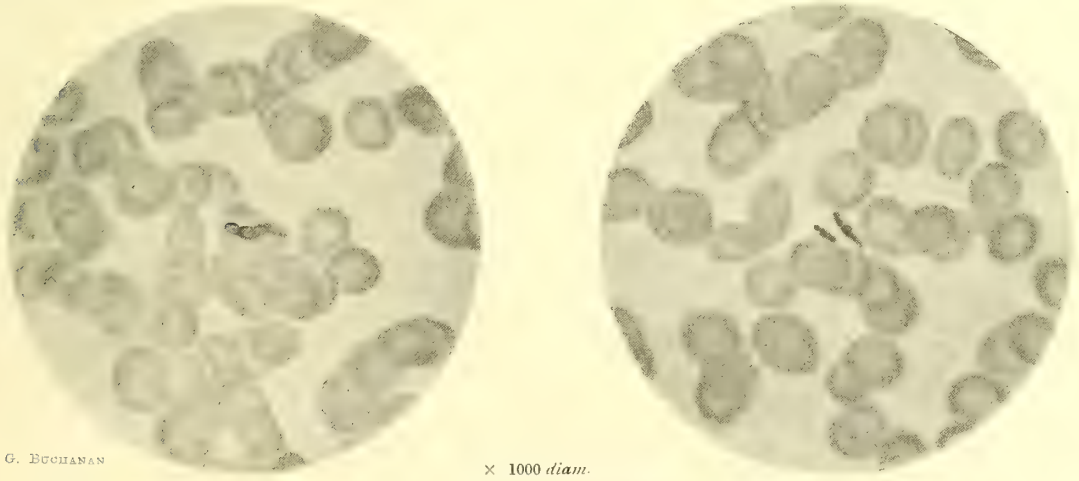
[6] Nicolle, C., and Manceaux, L. (September 25, 1910), "Recherches sur le bouton d'Orient—Cultures, Reproduction expérimentale, Immunisation." *Ann. de l'Inst. Past. Paris.*

Having seen Nicolle's paper, I then tried his special agar. At that time, not having invented the apparatus described on *pages* 107–108, I found it very difficult to prevent contamination by air organisms. The result was that though I obtained a partial development I was never able to secure flagellated forms. It occurred to me that, from the nature of the lesions, the parasites might be either obligatory or facultative anærobes, so I endeavoured to cultivate them under anærobic conditions, without any better result. Finally, as stated, the sores healed up and I was unable, owing to the disappearance of the parasites, to carry out any further work in this direction.

Possibility of different species of skin Leishmania

From a more detailed study of the morphology of the parasite I must confess that I am unable in any way to differentiate it from *L. tropica*, hence any special description is unnecessary. This agrees with the findings of Ferguson and Richards, but I do not think the question of species can be settled without knowledge of the cultural forms, the effects of inoculation and the carrier, the matter being on the same footing as animal trypanosomiasis. I note that Castellani and Chalmers[1] think it very probable that there are different species of skin Leishmania.

During the whole course of these cases the parasites found were characteristic save on one occasion, namely on January 2, 1910, when the contents of the right shoulder growth of Case I were examined. Films made from the face nodules and from a small accessory growth on this date were negative as regards the presence of parasites, but in the shoulder growth films, curious cockle-shaped bodies were found along with the spherical homogeneous blue bodies which are so characteristic of *Leishmania* infections and which may or may not be portions of the cytoplasm of fragmented host-cells (macrophages). It is difficult to say what these cockle-shaped bodies are. Do they represent a preflagellate stage of development or are they degeneration forms in a healing nodule? Again, may they not represent an accidental contamination by some form of yeast cell, considering that the shoulder growth had been frequently punctured? It is possible they are a form of Leishmania, and are evidence that the parasites in these nodules are a distinct species, but I am not inclined to subscribe to this hypothesis. Amongst them there were some



G. Buchanan

× 1000 *diam.*

Figs. 47 and 48.—Curious [redacted] bodies

"Cockle-shaped" bodies

bodies resembling the vermicules of hæmogregarines but much more minute. They vary in shape and size, some being cockle-like, others elongated. Their nuclei, or what may

[1] Castellani, A., and Chalmers, A. J. (1910), *Manual of Tropical Medicine*. London.

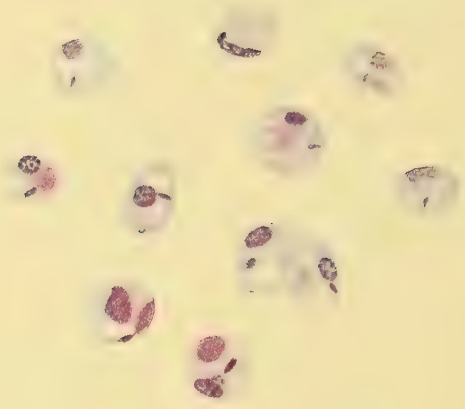
be called their nuclei, stain a very rich ruby-red by the Romanowsky method, and their general appearance is shown in Figs. 47 and 48.

In none of them is there any appearance of a rod-shaped blepharoplast, though some of the longer forms show, in addition to the nucleus (?), red chromatin granules rather like those met with in fusiform bacilli. Their cytoplasm stains a deep blue and in the long forms is distinctly granular. They are all extra-cellular and occur singly or in little groups. It is significant that no true Leishmania were present in the films. Perhaps these bodies scarcely merit attention, but it seems worth while mentioning their presence as it is difficult to place them and they might confuse other observers. On the whole I think they are some kind of yeast or fungus, but I did not make any attempt to cultivate them from the blood obtained by puncture of the growth.

As will be seen this research has had to be left incomplete, but it is hoped that in the future more cases of this peculiar condition may be seen, and that it may be possible to cultivate the causative organism, to carry out the necessary animal inoculations and to determine, no easy matter, the method of infection and transmission in man.

Meanwhile, the reader is referred to Captain Archibald's account of an allied cutaneous Leishmaniosis which it was possible to study in greater detail and which presents several points of interest (*vide page* 207).

PLATE XIII



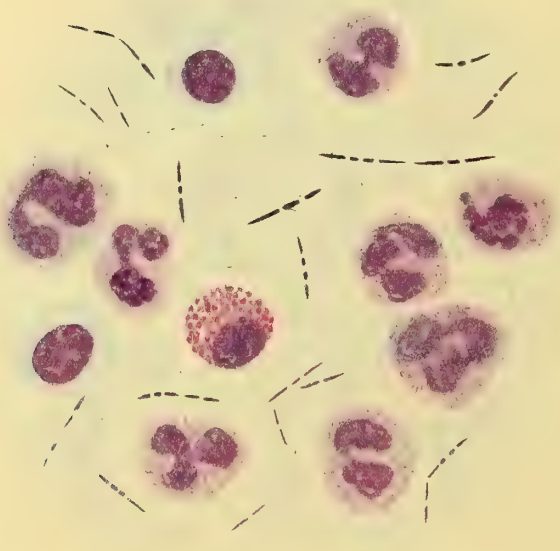
1

G. Buchanan

1. Leishmania tropica

Parasites from ulcer on forearm, showing developmental forms

Leishman Stain × 2000 *diam.*



2

R. D. Muir

2. Film from Ulcus tropicum

Smear showing spirochætes and fusiform bacilli

Leishman Stain × 1000 *diam.*

A Case of Parasitic Granuloma in which Developmental Forms of Leishmania tropica were Present

BY

Captain R. G. Archibald, M.B., R.A.M.C., attached E.A.

Pathologist and Assistant Bacteriologist

Wellcome Tropical Research Laboratories, Khartoum

Balfour and Thomson[1] recently described two cases of non-ulcerating oriental sore in which the lesions presented themselves as pink keloid-like growths.

In these growths the typical parasites of oriental sore were present and the name of Leishman nodules was suggested for them as a better form of nomenclature for this type of lesion. A few months ago a case which, in some points, resembled the cases described by Balfour and Thomson, was sent from the Khartoum Military Hospital to these Laboratories for microscopical investigation.

The history of the case was as follows:—

Ibrahim Bachit, aged about 30, a soldier in the 5th battalion, was admitted to the Khartoum Military Hospital suffering from various growths on the face, arms, body and legs. These growths had been in existence for a period of four months. There was nothing of singular importance either in the patient's history or in that of his family. He was a Nuba of good physique, with no previous history of syphilis and there was no enlargement of the spleen or liver.

Through the kindness of Captain R. J. C. Thompson, R.A.M.C., facilities were given for the purpose of studying the various skin lesions present in this case.

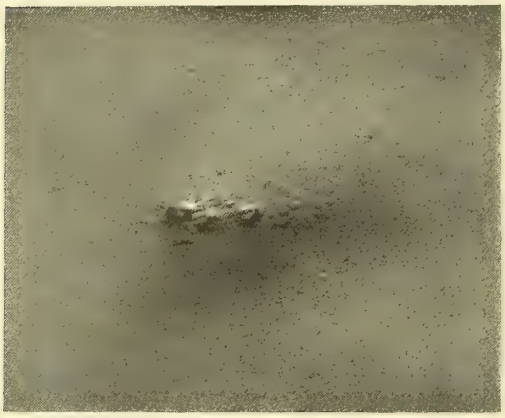
Appearance of the skin lesions

On examination, these skin lesions presented themselves in the form of slightly raised soft papules varying in size from 1/16 inch to $\frac{1}{4}$ inch (Figs. 49 and 50). They were situated for the most part on the arms and body, a few being present on the face and legs. One or two of the papules had become pustular in nature and were covered by a thin scab. On the flexor aspect of the right forearm there was also present a skin lesion which resembled in appearance a syphilide, and, situated behind the right external malleolus, there was, in addition, a small warty growth with an irregular surface.

According to the patient these multiple papular growths first appeared on the arm as small pink spots about the size of a pin's head, steadily growing till they had reached the size of a pea; their existence was not associated with any pain or irritation. Examination of them with a hand lens showed the presence of a pale pink centre over which the epidermis appeared to be stretched and thinned out. On palpating the individual papules a definite indurated margin could be felt; they were freely movable over the subjacent tissues. Around some of the papules there were present small pink pin-point elevations which were evidently secondary growths appearing around a primary one. There was no enlargement of the lymphatic glands either in the neck, axilla or groin. A fine glass pipette was inserted into the centre of each papule and the contents, consisting of blood, serous fluid, and a cheesy material, were blown on to a slide and stained by Leishman's stain. During this procedure it was quite evident that the coagulability of the blood was considerably increased.

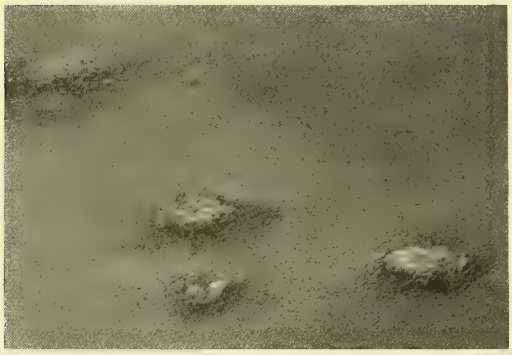
[1] Balfour, A., and Thomson, D. B. (January, 1910), "Two cases of non-ulcerating Oriental Sore, better termed Leishman nodules." *Transactions of the Society of Tropical Medicine and Hygiene.*

Microscopical examination of the stained specimens showed the presence of red blood corpuscles, granular debris, and a large number of eosinophile leucocytes. No Leishman bodies were in evidence, but, instead, there were present peculiar oval pale blue staining



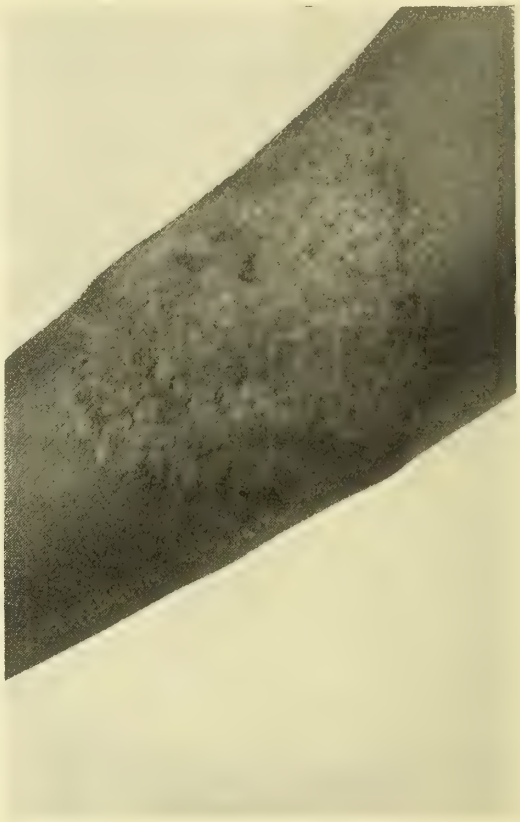
G. Buchanan

Fig. 49.—Papules on Chest



G. Buchanan

Fig. 50.—Papules on Abdomen



G. Buchanan

Fig. 51.—Skin lesion on Forearm (Ibrahim Bachit)

bodies about 3 μ in length; a few of these bodies contained a small amount of chromatin, but the majority were simply blue homogeneous looking cells. Similar "blue bodies" had previously been found in the peripheral blood and spleen smears of cases of kala-azar, and their presence in the contents of the papules aroused the suspicion that the case was one of Leishmaniosis, although no parasites were found in smears taken from them. The pustules were also punctured, but apart from the presence of diplococci nothing of additional interest was present. Attention was then directed to the ulcer on the flexor aspect of the forearm. The margins of this ulcer were punctured and smears made and stained. While examining these smears Captain Harvey, R.A.M.C., found Leishman bodies present. These bodies were oval in shape, and varied in size from 3 μ–4·8 μ in their longest diameter. Their average size was 3·4 μ. Stained by Leishman's modification of Romanowsky they were of a pale blue colour and showed a trophonucleus, a chromatin-staining kinetonucleus, and, in addition, a circular or oval body of a delicate pink colour, situated as a rule adjacent to and between the tropho- and kinetonucleus (Plate XIII., fig. 1). The nature of this delicate pink body it was difficult to determine, but it seemed to correspond to the description of the "eosin body" given by Rogers[1] in developmental

The "pink body"

[1] Rogers, L. (1908), *Fevers in the Tropics.*

forms of the Leishman parasite obtained from cases of kala-azar in India. Similar bodies have been obtained by me in cultures of the Leishman parasite obtained from cases of kala-azar in the Sudan; they represent, therefore, a cultural stage of the parasite in a local sore. Many of the parasites exhibited marked vacuolation. The kinetonucleus appeared either in the form of a dot or well-defined rod, which, in some of the largest parasites had segmented into two, or even three, parts; a further evidence of a developmental stage. Mitotic changes were present in some of the trophonuclei. All the films taken from the contents of the papules and from this sore showed the presence of a well-marked eosinophilia. The character of the sore which contained the Leishman bodies merits some description.

Character of the sore

In appearance it resembled a syphilide (Fig. 51), and was rather of the nature of a granuloma. It measured $3\frac{1}{2}$ by 3 inches and was limited by a sinuous margin, composed of confluent masses of papules similar to the discrete papules described on the other parts of the body.

The surface of this lesion was irregular and consisted of projecting masses of granulation tissue; some of these were covered by scaly scabs, underneath which a sero-purulent fluid was present. In smears taken from the centre of this ulcer a large number of diplococci were present, as well as a few degenerated Leishman bodies. Even in the margins of the ulcer but few parasites were present and these totally disappeared in two or three days.

Cultivation experiments on blood agar were failures. This was possibly due to the paucity of parasites present in the local lesion.

A differential blood count of blood taken from the peripheral zone of the granuloma was as follows:—

Polymorphonuclear leucocytes	41 %
Large mononuclears	19·3 %
Large lymphocytes	4 %
Small lymphocytes	7·4 %
Eosinophiles	27·7 %
Transitionals	·6 %

Local eosinophilia

There was evidently a well-marked eosinophilia, and an increase of the mononuclear leucocytes at the expense of the polymorphs and lymphocytes. On the same day a blood count, of blood taken from the tip of the finger, showed 5,436,000 red blood cells and 5,260 white blood cells. A differential blood count showed:—

Polymorphs	63·3 %
Mononuclear leucocytes	14 %
Large lymphocytes	6 %
Small lymphocytes	13 %
Eosinophiles	3·2 %
Transitionals	·5 %

This count showed that it was not far removed from the normal, except that the mononuclear leucocytes were somewhat increased. The fæces of the patient were also examined for the presence of intestinal parasites, but with negative results.

The papules were repeatedly punctured for the purpose of ascertaining whether parasites had yet appeared in them, but the results were negative.

Histo-pathology

Portions were excised from the edge of the granuloma and from several of the papules, and fixed by various methods, *viz.*, formol-alcohol, mercuric chloride, and alcohol. Sections were cut, and suitably stained for the purpose of demonstrating the presence of parasites and any pathological changes apparent.

Histological characters

The histological changes were more or less constant in them all, and were characterised by the presence of vertical epithelial columns extending downwards to varying depths as far as the dermis. These downgrowths of rete (Fig. 52) appeared to be very characteristic, and contained a few cell nests and isolated masses of cell infiltration. In the dermis there was present a very well-marked cell infiltration consisting of aggregations of cells of the large round cell type. Polymorphonuclear leucocytes and lymphocytes were also in evidence and a large number of eosinophile leucocytes. Scattered irregularly throughout the dermis were pale cellular areas which, under a high power, were seen to consist of collections of large mononuclear cells, many of which were vacuolated and lying in a homogeneous matrix. The sebaceous glands and hair follicles shared to a slight extent in this cellular infiltration. The large mononuclear leucocytes were carefully searched for the presence of phagocyted Leishman bodies but with negative results, nor were any free parasites present. Some of the sections showed the presence of bacilli and diploccocci.



Fig. 52.—Section of papule from case in which developmental forms of Leishman bodies were found

No parasites found in the papules

Some points of interest present themselves in this case, which, from a clinical point of view, differed somewhat from the cases of Leishman nodules described by Balfour and Thomson. In their cases the lesions were more of a keloid character and did not exhibit any tendency to ulceration. The pathological changes were more or less identical, and for this reason one feels inclined to consider the papular growths described in this case as evidence of Leishmaniosis although parasites were never found in them.

Ferguson and Richards[1] have recently described cases of parasitic granuloma in Lower Egypt. These observers met with two varieties, a flat and a warty form, and in many

[1] Ferguson, A. R., and Richards, O. (July, 1910), "Parasitic Granuloma." *Annals of Tropical Medicine and Parasitology.*

of their cases the parasites of Leishmania were either few or wholly absent from the lesions. From their description it seems that this case is identical, clinically and pathologically, with the cases met with in Lower Egypt. The warty type of growth appears to result from the effects of friction and pressure, a fact well brought out in this case by the warty nature of the growth situated behind the right external malleolus.

The chief point of interest in the case was the fact that Leishman bodies had undergone a certain amount of development in the granuloma. As evidence in favour of this was the large size of some of the parasites and the presence of the "eosin body" in their cell cytoplasm. No reference to this stage of development has been made by Row[1] in his recent and excellent work on the development of flagellated organisms from oriental sore, nor can one find any reference to it in the works of other observers.

Evidence of "development" of the parasites in the lesions

The well-marked local eosinophilia is difficult of explanation. Its existence is possibly brought about by local irritation or may possibly result from a local toxin.

The oval blue staining bodies that were found in smears made from the contents of the papules and from the granuloma have been constantly found in the peripheral blood and spleen smears of cases of kala-azar in the Sudan. One is rather inclined to look upon them as indicative of degenerative blood changes rather than representing degenerated forms of Leishman parasite. It is difficult to give a definite opinion concerning their nature. In all probability they represent degenerated leucocytes or detached portions of macrophages.

The facts to which I have called attention may be thus summarised :—

1. The existence of a skin lesion in the Sudan allied to parasitic granuloma in which developmental forms of Leishman bodies were present.

2. The lesions show themselves clinically under two forms, one a slightly raised, smooth patch with a pink centre which may or may not undergo ulceration; the other a warty form of growth resulting from friction or pressure.

3. The histopathological changes in these lesions consist in an increase of the rete Malpighi with a marked mononuclear cell infiltration of the subcutaneous tissues.

4. The increased coagulability of the blood in the lesions.

5. The presence of a marked local eosinophilia.

[1] Row, R. (1909), "Development of flagellated organisms from the parasite of Oriental Sore." *Transactions of the Bombay Medical Congress.*



W Beam

Fig. 53.—Skin lesions on lower jaw
(Egyptian officer)



W Beam

Fig. 54.—Skin lesions on wrist
(Egyptian officer)



W. Beam

Fig. 55.—Skin lesions on neck
(Egyptian soldier)



W. Beam

Fig. 56.—Skin lesions on wrist
(Egyptian soldier)

Two Cases of Veldt Sore Treated by Autogenous Vaccines

by

The Director

In the *British Medical Journal* for January 22, 1910, there appeared two interesting papers, the first[1] dealing with the bacterial vaccine treatment of a series of cases of staphylococcic infection, the second[2] with the vaccine-therapy of cases of ulceration of the face and fauces. The experience of the author of the first paper led him to the following conclusions:—

1. That vaccine is of great service in most staphylococcic infections.
2. That staphylococcic skin lesions, however chronic, are curable by its means.
3. That in many cases estimation of the opsonic index is unnecessary.
4. That the best results are only obtained by the use of autogenous vaccines.

Since the paper appeared, an important series of meetings of the Royal Society of Medicine has taken place whereat all the pros and cons of vaccine-therapy have been fully discussed, and to judge by the published proceedings,[3] the views of Dr. Clarke Begg have received confirmation.

The second paper dealt with cases of mixed infection in some ways closely resembling those about to be described. I have thought it well to put these latter on record as, naturally enough, vaccine-therapy has not yet been greatly exploited in tropical countries, partly from the lack of facilities afforded by laboratories, and partly because commercial vaccines do not keep well in hot climates. I have little doubt that a large number of bacterial lesions in the Tropics, and especially skin lesions, will eventually be successfully treated by appropriate vaccines, and certainly, in the cases to be described, this mode of treatment appeared to act like a charm.

In February, 1910, I was informed by the Senior Medical Officer, Khartoum District, that a considerable number of men in the Egyptian cavalry, at Shendi, were suffering from cutaneous ulcers, and that the epidemic, for the disease was undoubtedly contagious, had spread to the civil population. The Senior Medical Officer visited the cavalry lines and sent me slides of the discharge from the sores from fourteen different cases. Although a few of these contained streptococci and bacilli of different kinds, two chief bacterial conditions were easily recognised. One set of cases showed a diplococcal infection, the other, a diplococcal infection together with an infection by diphtheroid bacilli.

Mixed infection by a diphtheroid bacillus and a diplococcus

The diplococci were found free and in the polymorphs, a few being also present in the mononuclear leucocytes. The diphtheroid bacilli were for the most part free.

At a later period, two cases were sent me for examination and the following histories were kindly supplied by Mulazim Awal Iskander Eff. Sarkis, of the Egyptian Medical Corps.

CASE I.—Mulazim Tani A. D., a young officer in the Egyptian cavalry.

Family history of no special importance.

[1] Begg, A. C. (January 22, 1910), "Observations on the vaccine treatment of a series of cases of staphylococcic infections."—*British Medical Journal.*

[2] MacWatters, J. C. (January 22, 1910), "Ulceration of the face and fauces treated by bacterial vaccines." —*British Medical Journal.*

[3] Wright, A. E. and Others (October, 1910), "Vaccine-Therapy: its administration, value and limitations." —*Proceedings of the Royal Society of Medicine.* Vol. III., No. 9. Supplement.

Previous illnesses.—None. *General condition.*—Satisfactory.

Present disease.—Two months ago two small papules appeared on his right wrist. A week later, another couple appeared on his left cheek. All began to enlarge and redden, and, within a month, presented the appearance of large pustules. Crusts formed upon them which the patient picked off and a purulent discharge was established. They were treated by washing with sublimate solution and by the application of silver nitrate, but without any beneficial result.

CASE II.—Nafar No. 4841. Moh. Hussien Eid, 2nd Batt. Egyptian cavalry. Age 21 years.

Family history of no special importance.

Previous illnesses—not stated.

General condition—satisfactory, a healthy man of good physique.

Present disease—Began three months ago at Shendi. Small pimples, about the size of peas, appeared on his left forearm. These were at first red in colour, but afterwards became reddish-grey and scaly. They were itchy. After a few weeks, more spots made their appearance on his forearm, lower lip, neck and chest. These enlarged, indurated and fissured. Some broke down and discharged. Men in the same barrack-room were similarly affected.

On seeing these sores I recognised the condition as being, in all probability, one with which I used to be very familiar in South Africa—namely, veldt sores. The fact that the men, primarily affected, were associated with horses, tends to confirm this supposition. In both cases (Figs. 53, 54, 55, 56) the ulcers were of the same type, with indurated, inflamed, undermined edges, and flat, dense and fissured crusts. The pus was thick and yellow or greenish-yellow, but not offensive.

The officer (Figs. 53, 54) was in a state of considerable mental distress owing to the chronicity of his disorder and its disfiguring effect.

The discharge from the sores in Case I. contained almost no organisms but diplococci, which were present in great numbers. On culture, the organism proved to be *Staphylococcus pyogenes albus*.

Successful treatment by autogenous vaccine

I prepared a vaccine, and, on February 12, a dose of 250,000,000 staphylococci was given, the only other treatment being the local lavage and dressing recommended by MacWatters,[1] namely, frequent bathings with a lotion containing sodium citrate, 1·5 per cent., with common salt, 20 per cent.; together with small dressings of lint soaked in the same lotion during the night. This is said to prevent clotting of the exuding lymph and to promote by osmosis a flow of the highly opsonised lymph through the infected tissues.

There was an almost immediate improvement which was rapid and progressive. By February 24, the ulcers presented the appearance of healthy healing sores. The crusts were gone, the surfaces healthy and granulating, the edges were softer and had become shelving, while a bluish-white film of young epithelium everywhere surrounded the healing wounds. The opsonic index was not determined, partly because I was much too busy to attempt it and partly because it appeared unnecessary.

On February 26, a second dose of 500,000,000 was given, and, by March 1, the sores had completely healed, local induration being the only sign remaining.

On March 5, the patient was discharged from hospital and returned to duty.

It is true that the early treatment had neither been very extensive nor very thorough, but when one remembers how obstinate cases of veldt sore used to be and the extensive pathological changes in the case in question, one can only conclude that the rapid and complete cure was due in very large measure to the vaccine-therapy. Certainly I have

[1] *loc. cit.*

never seen ulcers of this kind heal up so well or so quickly, and the patient's astonishment and pleasure were very gratifying.

Films made from the ulcers in Case II. showed diplococci and diphtheroid bacilli (Figs. 57 and 58), the former being the more numerous. The cocci yielded an orange growth on agar and proved to be *S. pyogenes aureus*, which, however, is probably only a variant of *albus* and *citreus*, all being forms of *S. pyogenes*.

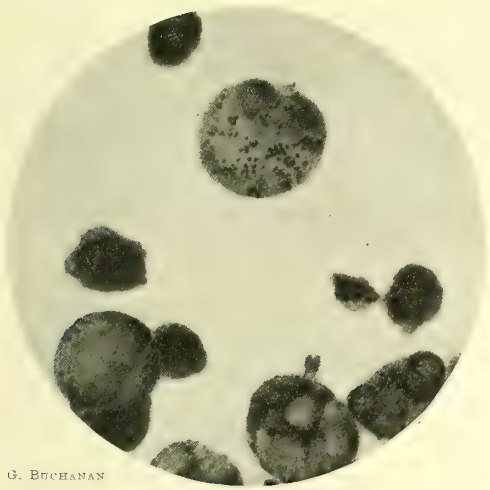


Fig. 57.—Case II., film from ulcer showing diplococci

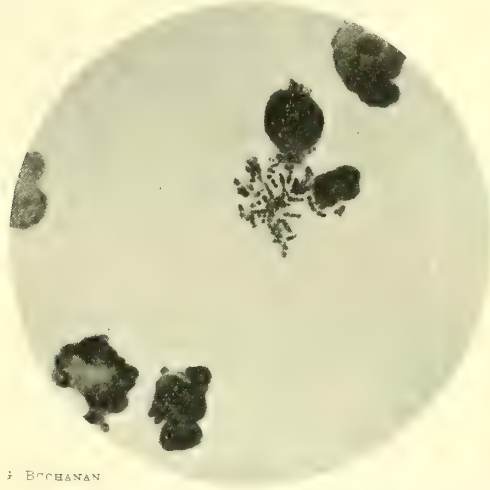


Fig. 58.—Case II., film from ulcer showing diphtheroid bacilli

A staphylococcic vaccine was first prepared, and on February 20 he was given 250,000,000 with the same local treatment. Improvement was slower in starting in this case, but had commenced by February 24.

On February 24, the ulcer on the left forearm was found to be still undermined at the edges but to be clean and dry. The other two sores were still flat and crusted as was the ulcer on the chest from which yellow pus was exuding. Its periphery was angry and inflamed. Both diplococcic and diphtheroid bacilli still persisted in the discharge.

In consequence, the bacilli were isolated, cultivated, and a vaccine prepared from them in the usual way.

Use of a mixed vaccine

On March 1, doses of 500,000,000 staphylococci and 10,000,000 bacilli were issued and administered by hypodermic injection on March 3.

By March 8, a very notable change had occurred, apparently as a direct result of the double inoculation. The large ulcer on the forearm was quite clean and nearly healed, though slightly sodden in appearance. It was no longer undermined at the edges and the discharge had practically ceased. The two small ulcers had completely healed up. The large sore on the chest was healed throughout three-quarters of its area, a white scaly scar having formed. The remaining quarter was covered with a thin skin or film which was of a yellowish-green colour, probably as a result of a thin layer of dried pus underneath it. Its edges were puckering and healing and the inflamed areola was much less marked. The discharge had ceased.

A few days thereafter, scars had taken the place of all the ulcers, and shortly afterwards the patient was discharged well, and returned to duty.

The two cases present an interesting contrast, as, for the first, a single vaccine sufficed, while for the second, a double one was required.

I am indebted to Captains Mackenzie and Cassidy, of the Egyptian Medical Service, for carrying out the special treatment and furnishing me with material and notes.



G. Buchanan

Fig. 59.—Leucoderma (front view)



G. Buchanan

Fig. 60.—Leucoderma, same case
White Patches and Scaly Eruption



G. Buchanan

Fig. 61.—Leucoderma (back view)

Ulcus Tropicum

BY

The Director

Plate XIII., fig. 2

This is another form of skin lesion which does not appear to be very common in the Sudan. To judge by specimens received it would seem to be more frequent on the Red Sea coast than elsewhere. It need only be mentioned, as the microscopic findings are exactly like those detailed and figured by Keysselitz and Mayer[1] in their admirable paper. The cause of the disease would appear, in all probability, to be the spirochæte described by Prowazek under the name of *S. schaudinni*. Its frequent association with fusiform bacilli is interesting. In the few cases seen in the Sudan this symbiosis has always been encountered.

Probable cause of disease

Castellani and Chalmers[2] give an excellent account of the condition, and I note they say that it is particularly common in certain islands of the Red Sea.

Leucoderma

BY

The Director

In our First Review Supplement attention was directed to this condition in the Tropics. Recently, a paper by Sir Jonathan Hutchinson[3] appeared in which he discusses the subject fully and which has led the Editor of the *Indian Medical Gazette* to ask for information regarding its prevalence amongst Indians. It is said to be spreading amongst the Parsees.

The disease occurs in the Sudan and appears to be associated with syphilis. Figs. 59–61 are photographs of a case in a syphilitic Sudanese soldier from the Lado District, Upper White Nile.

In addition to the white patches there was a serpiginous, scaly eruption the spreading edges of which looked as though coated with hoar-frost. These distinguished the condition from Pinta, as did the absence of any fungus in samples of the scales examined by Captain Archibald. A few cases of Pinta have been recorded from Egypt so it is necessary not to confound the two conditions. The photographs were taken by Mr. Buchanan with the kind permission of Captain Burney, R.A.M.C., who had charge of the case. Some leucoderma cases might also have to be distinguished from anæsthetic leprosy. The etiology of true tropical leucoderma is apparently quite unknown, but it is regarded as a trophoneurosis. In this case, of course, the syphilis may only be an accompanying condition, but the association of the two skin lesions is suggestive.

Serpiginous, scaly eruption

[1] Keysselitz, G., and Mayer, M. (1909), "Über das Ulcus tropicum." *Archiv. für Schiffs- und Tropen-Hygiene.*

[2] Castellani, A., and Chalmers, A. J. (1910), *Manual of Tropical Medicine*, London, *pp.* 1118–1122.

[3] Hutchinson, J. (September 1910), "Leucoderma in Dark Races."—*Polyclinic*, quoted in *Indian Medical Gazette*, December, 1910.

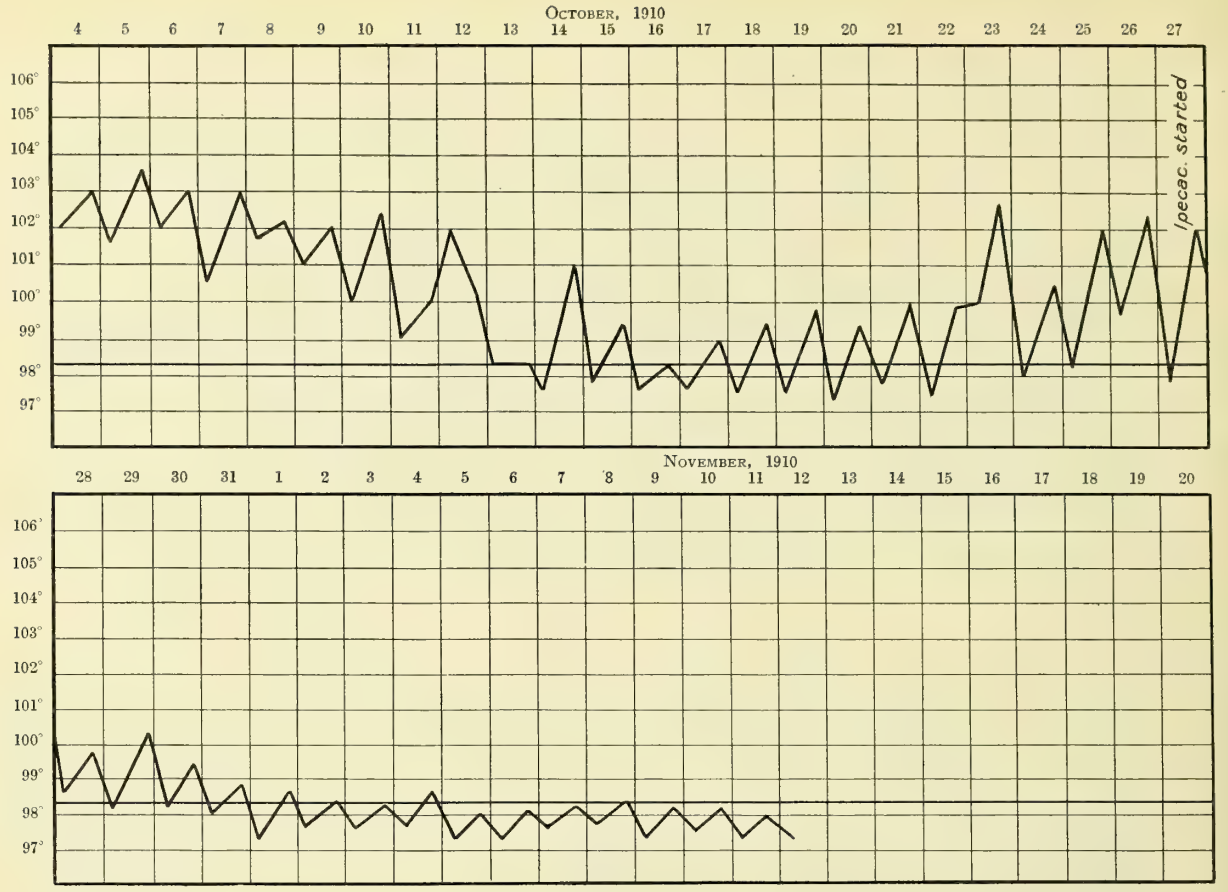


Fig. 62.—Chart 1, Hepatitis

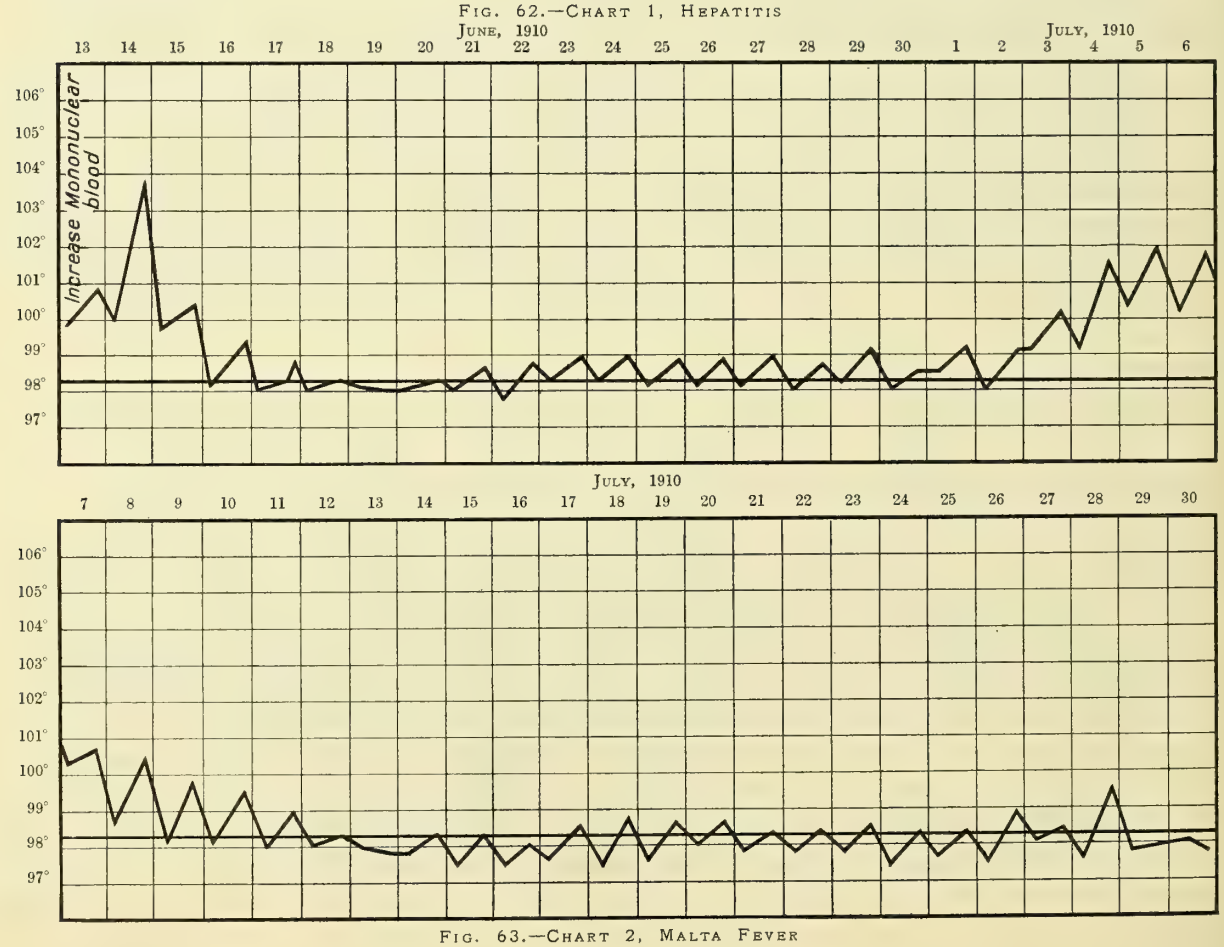


Fig. 63.—Chart 2, Malta Fever

Fevers in the Sudan

Preliminary Note

by

The Director

No subject is of greater importance in the Sudan than that which gives this paper its title. In some respects, also, there is no subject about which there is more to be learned. It is true that in the case of a certain number of febrile disorders in the Sudan we are on sure ground as, for example, enteric fever, the paratyphoid fevers, malaria, spirochætosis, Malta fever, the febrile dysenteries, the fever due to hepatitis, cerebro-spinal fever, diphtheria, influenza and pneumonia. In such cases we know the cause and can treat the condition with more or less chance of success. What is better, we can very often prevent such diseases occurring or check their spread. A second class exists where we know either the cause or the method of infection and can do something towards preventing them, but where, so far, treatment is of little avail. As examples may be mentioned trypanosoma fever, kala-azar, phlebotomus fever, dengue, measles, variola and varicella. Finally, there comes a group where it is often difficult to make a diagnosis, where the condition may be trivial or of great gravity, where the explanation, when obtained, may be simple in the extreme but where such explanation can only be secured by careful clinical and laboratory work and by following out the synoptic methods so ably set forth by Sutherland[1] of Lahore. In this class come the ptomaine poisonings, the obscure tubercular processes, especially general miliary tuberculosis, the fever associated with visceral syphilis, fevers due to infection with metazoan parasites, fever due to old malaria infection, cases of idiopathic splenomegaly, certain septicæmic conditions, paracolon and atypical paratyphoid fevers, *B. coli* infections of the urinary tract and the febrile disorders commonly put down to the effects of sun, chill or fatigue and about which we know very little.

Classes of fevers in the Tropics

There is no need to deal here at any length with the first class. So far as one knows, with the single exception of diphtheria, the facts gathered concerning them are in accord with those which have been set forth regarding them in India and elsewhere. Diphtheria and spirochætosis receive special chapters to themselves in this Report and nearly all the others have claimed attention, either in previous Reports or in our Second Review Supplement.

The pre-suppurative stage of hepatitis has, however, not been mentioned, but, thanks to the work of Rogers[2] in India, one has been on the look-out for cases of this kind. Quite recently one was encountered in the Egyptian Army Hospital, Khartoum, under the care of Captain A. G. Cummins, R.A.M.C. He had been running a temperature since admission, 23 days previously (*Chart* I.) Blood examination was negative, so far as parasites were concerned, and his stools showed nothing abnormal. Captain Cummins had detected a slight increase of liver dullness and there was some pain and tenderness in the hepatic region. On the right side there was also a transitory pleural rub leading to a suspicion of pneumonia. There was no shoulder pain and only slight insomnia. At first the case was regarded as an indefinite type of pneumonia but, as will be seen

Pre-suppurative stage of hepatitis

[1] Sutherland, D. W. (September 12, 1908), "The Differential Diagnosis of Fevers." *Lancet*, London.

[2] Rogers, Leonard (1910), *Fevers in the Tropics*, Second Edition.

from the chart, the exhibition of ipecacuanha both cleared up the diagnosis and cured the condition.

An abnormal chart in Malta Fever

Chart 2 is interesting as showing a somewhat abnormal temperature in a case of Malta fever. The case was one of laboratory infection and the agglutination reaction marked, but fever was never a very marked symptom though most of the usual characteristics of a mild case were present. We know that the blood in cases of typhus fever will agglutinate *M. melitensis*, and it is quite possible that other fevers exist which will give this reaction. Otherwise, it is difficult to explain the occurrence of two isolated cases in Khartoum, which had provisionally to be called Malta fever, but which may have been something quite distinct and hitherto unrecognised.*

Turning to the second class, a few words may be said regarding phlebotomus fever. Its existence in the Sudan has not yet been definitely proved, but I have good reason to believe it occurs, and indeed I am now strongly disposed to think that the short and sharp fever from which I myself suffered in Khartoum some years ago, and of which mention is made on *page* 69 of our First Review Supplement, was of this nature. Mr. King, also, after being badly bitten by *Phlebotomus* at Berber on May 27, 28 and 29, 1910, developed on June 3 a fever of short duration characterised by injected conjunctivæ and severe pains in the limbs and back which strongly suggested this condition. Two species of *Phlebotomus*, namely *P. papatisii* and *P.* sp. incert. are known to occur in the Sudan, while it is certain that a short fever of from four to seven days' duration is not uncommon. The fact that "sand-fly" fever is prevalent in Egypt also makes it probable that it occurs in the Sudan. In Khartoum, it was made notifiable some time ago but, so far, no case has been reported. I am inclined to think that *Phlebotomus* flies are not so common in Khartoum all the year round as they used to be. One hears fewer complaints regarding them and certainly encounters them much less frequently than used to be the case. In the summer months, however, they are still in evidence in certain parts of the town but their infectivity cannot be great or we would have many more attacks of what is known popularly in Malta as "Pink-eye."

"Sand-fly" fever

Dengue is a fever of much interest, but here I would only refer to the remarks on the differential leucocyte count in cases of this disease which appeared in our First Review Supplement (*pages* 37 *and* 38). The leucopenia, the diminution in polymorphs, the early and great small lymphocyte increase, the late large lymphocyte increase and the final increase of large mononuclears, are sufficiently characteristic when taken along with the symptoms and the negative blood findings as regards hæmatozoa.

It may here be said that for clinical work the instructions of Rogers for carrying out the differential leucocyte count, as set forth in the Second Edition of his book on *Fevers in the Tropics*, should be read and, I think, adopted.

It is, however, Class III. which possesses for us the greatest interest. Fever is not always a feature of ptomaine poisoning but it may be in evidence and cause a difficulty. As a rule the history and symptoms guide one to a diagnosis, but I confess it may be impossible to say whether a case of what we may call "toxic enteritis" is to be attributed to the effect of ptomaines or not.

Toxic enteritis

Here is the record and temperature chart (No. 3) of a rapid and fatal case in a young European adult which came under the notice of Captain Archibald and in which there was absolutely no history pointing to the ingestion of ptomaines. One can only term it a case of obscure toxic enteritis.

* It is also well to remember that Nègre has recently shown that, within certain limits, some normal sera will agglutinate the micrococcus. *C. R. Soc. Biol.*, December 17, 1910.

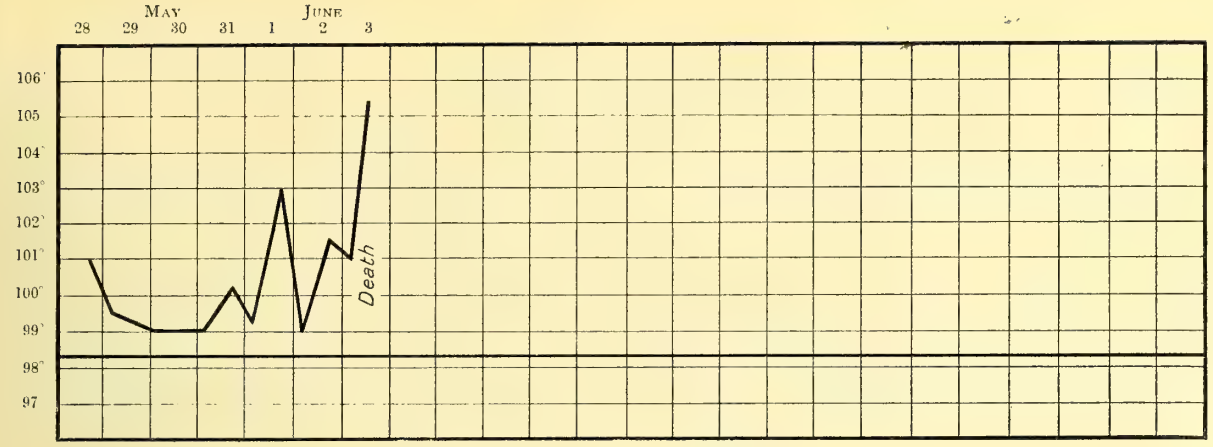


Fig. 64.—Chart 3, Toxic Enteritis (?)

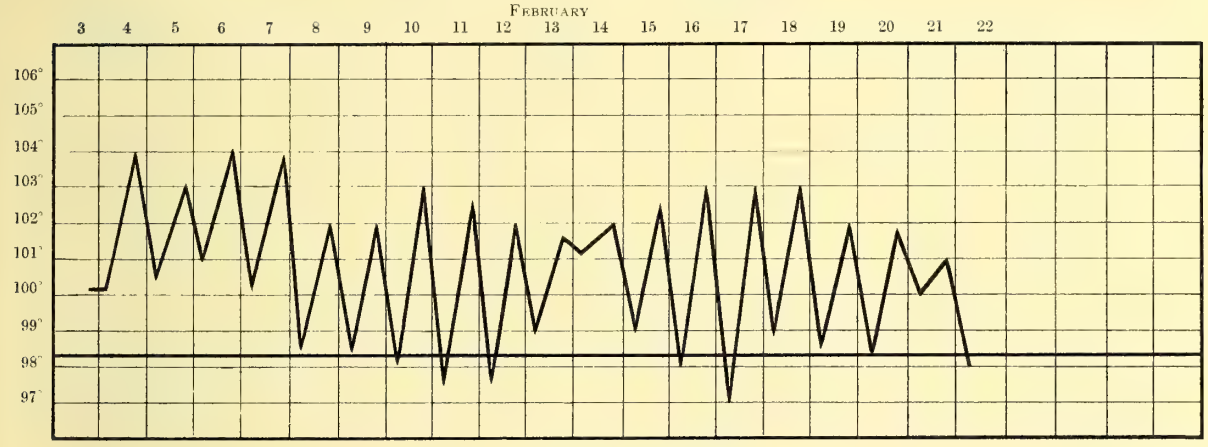
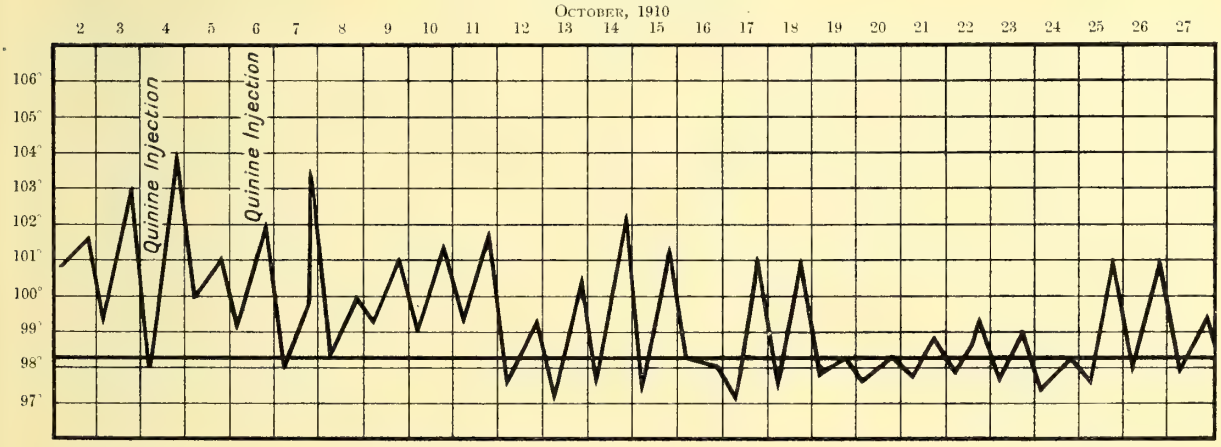


Fig. 65.—Chart 4, Tuberculosis



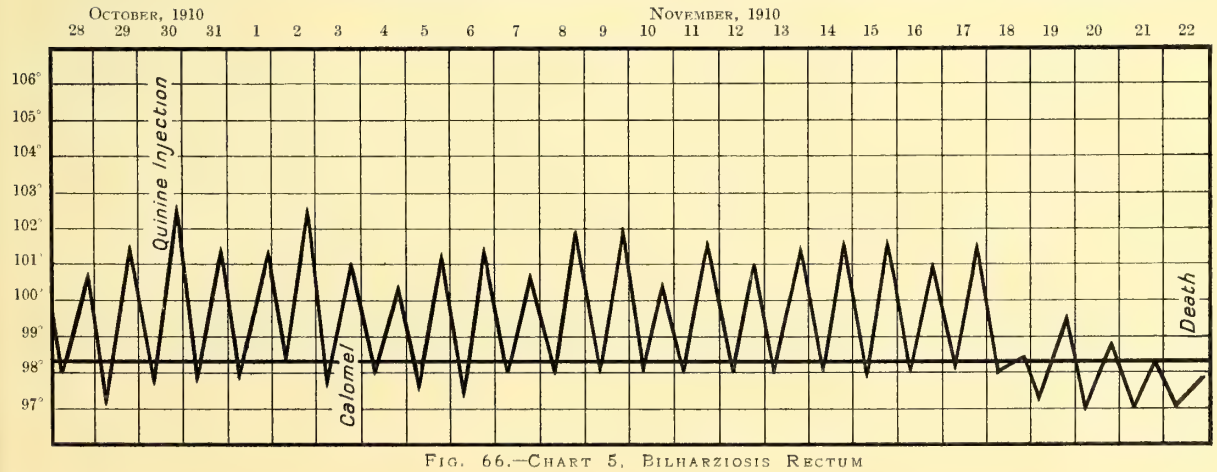


Fig. 66.—Chart 5, Bilharziosis Rectum

"*Friday, May* 28. Complained of headache and feeling generally unwell—tongue was very furred and conjunctivæ slightly congested. Temperature 101° F. On palpation, no area of tenderness over abdomen. Bowels had not moved for two days. Calomel, grs. iii., given. Patient in bed, milk diet. Blood films taken.

Notes of the case

"*Saturday.* Temperature 99·5° F. in the morning. Felt better. Bowels had not moved. A soap and water enema administered in the afternoon with good effect. Headache had disappeared. Blood examination for malaria negative. A careful examination of heart, lungs and abdomen was carried out but nothing abnormal was present.

"*Sunday.* Temperature 99° F. Patient felt very much better. Complained of feeling tired but had no pain or symptoms.

"*Monday.* Temperature 99° F. in the morning, 100·2° F. in the evening. Feeling of lassitude. No subjective or objective phenomena observed. Calomel, grs. iii., again given.

"*Tuesday.* Temperature 99·2° F. Felt better, still a little tired, but wanted to attend to his duties.

"*Tuesday afternoon.* Patient at four o'clock was extremely drowsy, did not seem inclined to answer questions. Temperature 103° F. Face somewhat cyanosed. Conjunctivæ injected. Tongue furred, breath foul. Had some diarrhœa in the afternoon. Patient removed to hospital. General condition improved.

"*Wednesday.* Mid-day, patient collapsed and infusion was carried out. Rallied and died on Thursday morning.

"Post mortem at 5 o'clock in the afternoon showed:—Lungs and heart normal. Intestine in region of ileum marked by congested patches (Enteritis) not related to lymphoid structures of gut. No ulcers. Spleen was slightly congested, not enlarged. Liver normal. Cultures taken but contaminated with post mortem bacilli. No pathogenic organism isolated."

Tuberculosis

Possibly cases of miliary tuberculosis in Egyptians or Sudanese are the most puzzling of all. As shown post mortem, a man may be riddled, so to speak, with tubercle; his lungs, liver and spleen full of nodules and foci of the disease and yet, unless his brain or his intestines are affected as well, he may exhibit no physical signs at all and the spiky fever chart, with weakness and emaciation, remain the prominent features of his case. In more civilised countries the various tuberculin tests would clear up the diagnosis in most instances, but here the differential blood count is often of most service, a marked lymphocytosis arousing one's suspicions. I think that in any case of obstinate and long-continued fever (*vide Chart* 4) it is well to bear the possibility of tuberculosis in mind and to remember that tuberculosis may complicate other febrile disorders. One is too apt to look for a single cause and to forget that two or more pathological states may act concurrently in elevating the temperature and in producing complex and confusing clinical pictures. So far the condition of Acute Tuberculous Fever described from India by Roberts and Bhandarkar[1] has not been encountered, but I do not know that it has been very persistently looked for in the Sudan. I once suspected I had met with a case, especially as acid-fast bacilli were present in the urine, but it turned out to be a pyelitis due to *B. coli* and the mere presence of acid-fast bacilli in the urine means nothing, for Philibert[2] has shown that some 13 species exist normally in that secretion. One must not overlook the possibility of the occurrence

[1] Roberts, J. R., and Bhandarkar, R.S.P.R. (February, 1908), "Preliminary Note on the Existence of an Acute Tuberculous Fever, in India, which has been confused with Continuous and Remittent Fevers."—*British Medical Journal*, p. 377.

[2] Philibert, André, *Les pseudo-bacillus acido-résistants.* Paris, 1908.

of Castellani's low, intermittent, non-malarial fever, though, so far as I know, it has never been definitely diagnosed in the Sudan. I have, however, seen cases resembling it. (*Vide Manual of Tropical Medicine*, Castellani and Chalmers, 1910, *page* 788.)

Syphilis

Now that we know that syphilis is a form of chronic spirochætosis, subject to acute or subacute exacerbations, it is not surprising that a prolonged fever of syphilitic origin may occur. This is specially true of visceral syphilis, and Hermann[1] has recently recorded a case of the kind. It is advisable, therefore, in doubtful cases to exhibit mercury and potassium iodide or possibly even "606," a procedure which may clear up a puzzling condition in a very short space, and at the same time may prevent the occurrence of symptoms which, while they may make the diagnosis certain, may also be distressing or dangerous to the patient.

Until recently the part played by helminths in producing fevers in the Tropics has not, I think, been sufficiently recognised.

Without trespassing on the functions of our Review Supplement, I may yet mention that Castellani[2] has drawn timely attention to the febrile condition in Ankylostomiasis, while in India several papers have appeared dealing with "Round Worm Fever." In any case of obstinate and obscure fever the fæces should be systematically examined.

Fevers caused by helminths

Here is the chart (5) from a case which, post mortem, showed marked Schistosomum lesions in the sigmoid flexure and the fæces of which were full of lateral-spined eggs. The chronic bilharziosis was apparently the sole cause of a prolonged elevation of temperature extending, almost without remission, over a period of 52 days, between the date of admission and the date on which death occurred. Malaria was excluded. Correct diagnosis in such a case can, unfortunately, rarely lead to effective treatment. Eosinophilia, often marked, is frequently the first guide to its true nature.

Fever due to old malarial infection, when there are no parasites to be found in the blood, is sometimes difficult to diagnose. A big spleen and a large mononuclear increase occur in other conditions, while, in the Sudan, pigment-containing white cells are distinctly rare in the peripheral blood. History goes for something, but what chiefly helps is to enumerate the red and white cells, especially the latter, so as to exclude leucopenia and to find out the relative count. Of course splenic puncture may clear things up, but it is not perhaps always justifiable unless, indeed, one is excluding kala-azar, and the blood state does not forbid the operation. In some cases only prolonged and efficient treatment drives one to the conclusion that the case was malarial in origin, and it is quite true that treatment is often as much a diagnostic as a curative agent in this class of illness.

Idiopathic tropical splenomegaly

Of still greater interest are cases of what, for want of a better term, is called idiopathic tropical splenomegaly, where a patient suffers from constant fever, marked splenic enlargement, some increase in the hepatic dulness, loses flesh and may look for all the world like a kala-azar case.

I have seen at least two examples in Europeans and the condition has, of course, been recorded from the Philippines[3] and India.[4] One case was in a British Officer from the Blue Nile. Malaria was excluded and the symptoms, clinical signs and temperature were exactly like those of a moderately severe case of kala-azar. Liver puncture was performed but no Leishman-Donovan bodies were found. There was marked degeneration

[1] Hermann, "Klinisch Anatomischer Beitrag zur Pathogenese des Viszeralen Luesfiebers." *Med. Klin. Berlin*, 1910, VI, 1015-1017.

[2] Castellani, A. (September 1, 1910), "Ankylostomiasis."—*Journal of Tropical Medicine and Hygiene.*

[3] Woolley, Paul G. (June 1906), "Tropical Febrile Splenomegaly."—*Philippine Journal of Science*, Vol. I.

[4] Faichnie, N., and Bond, J. H. R. (September, 1910), "Long-continued Fever with Marked Enlargement of the Spleen Cured by the Use of Senega."—*Journal Royal Army Medical Corps*, Vol. XV., No. 3.

of the leucocytes which showed chromatolysis. Major Howard Ensor, D.S.O., has very kindly furnished me with some notes on the case and with a copy of the temperature chart (No. 6) which was kept in Khartoum. Unfortunately the previous record is not available. The fever began early in September, 1910, and was treated by quinine without benefit. Blood examinations made on several occasions proved negative for malaria. Early in October the patient was sent to Khartoum as a possible case of kala-azar. The spleen was found to be much enlarged and very hard in consistence. There was no pain or tenderness in the splenic region. The liver had not appreciably increased in size. There was a history of old attacks of rheumatic fever, but no cardiac lesion was present. Neither Major Ensor nor myself could find malarial parasites in the peripheral blood. Quinine, however, was given together with salicylate of soda. A blood count on October 10 gave 5,320,000 reds and only 2000 whites. A confirmatory count gave practically the same result, proving that marked leucopenia was present. Next day liver puncture was performed with the negative result stated on *page* 223. Under the treatment mentioned, the temperature fell, still, however, reaching over 99° F. at night. The condition of the spleen remained unchanged. A blood count on October 16 showed the leucopenia to have somewhat diminished, there being 3,200 white cells. A second liver puncture gave negative results, as did frequent examinations of the peripheral blood. As the patient was improving Major Ensor did not think that splenic puncture was justifiable. He put the patient on his senega treatment, which apparently speedily reduced the spleen to normal size, abolished the leucopenia, eventually even inducing a slight leucocytosis (13,000 whites), and cured the patient. I can only say that both Major Ensor and myself regarded the case as one of early kala-azar, but as no parasites were found and splenic puncture was not performed a definite diagnosis is not possible.

Successful treatment by senega

The other example was also in a British Officer resident in Khartoum, but was not quite so like kala-azar. Apart from the curious fever (*Chart* 7), the chief symptom was a very great and rapid enlargement of the spleen which, by January 31, reached to half way between the umbilicus and Poupart's ligament. (Note by Major J. C. Jameson, R.A.M.C.). No malarial parasites were found in his blood and the differential count showed a marked lymphocyte increase, there being 34 per cent. of large and 21 per cent. of small lymphocytes. Some cells resembling myelocytes were also present. I feared the case would turn out to be a leukæmia. Under quinine treatment, however, improvement speedily occurred. Lack of space does not permit a longer record of this curious case. I believe it was a splenomegaly due to some toxin absorbed from the intestinal tract, though it may have been a peculiar attack of malaria. Nowadays, perhaps, an examination of the urine for pigment by Urriola's method might have cleared up the condition.

Mention may perhaps be made of septicæmic fever. This is not always easy to diagnose and, indeed, septicæmia, sapræmia and pyæmia are apt to be forgotten in the Sudan where one is on the look-out chiefly for malaria, enteric fever and kala-azar. Here is a case which seems worth recording as the temperature chart (No. 8) is interesting. The following notes are those kindly sent me by Dr. S. A. Saigh, the Medical Officer at Halfa, who also sent the chart along with them and who, I think, took a correct view of the case.—

Fevers of pyogenic origin

"*Previous History.* Patient complains of having had attacks of pain in the left side, coming on about every four months and lasting 24 hours; he has had these attacks since his childhood or, at least, as far back as he can remember, and they were always accompanied with fever.

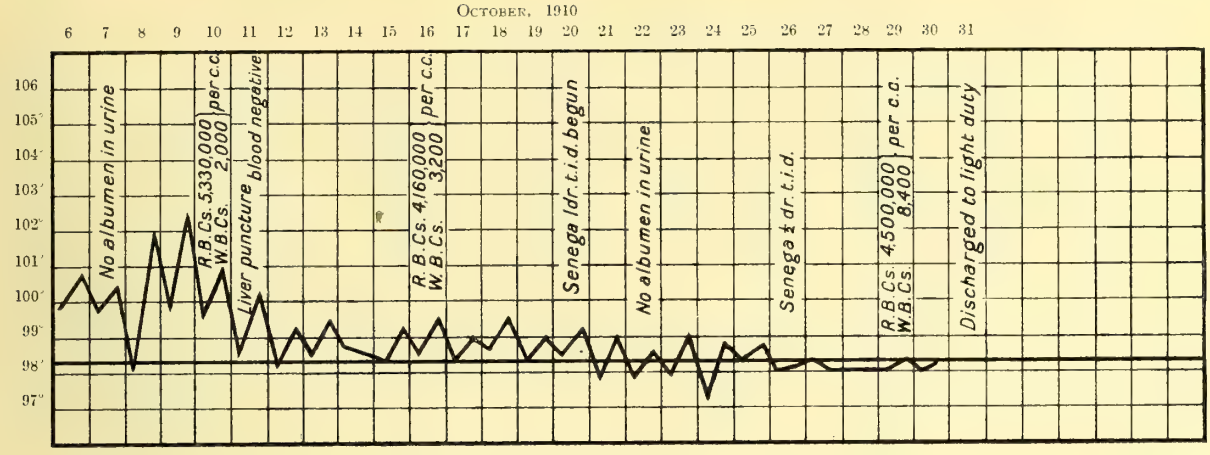


Fig. 67.—Chart 6, Idiopathic Tropical Splenomegaly

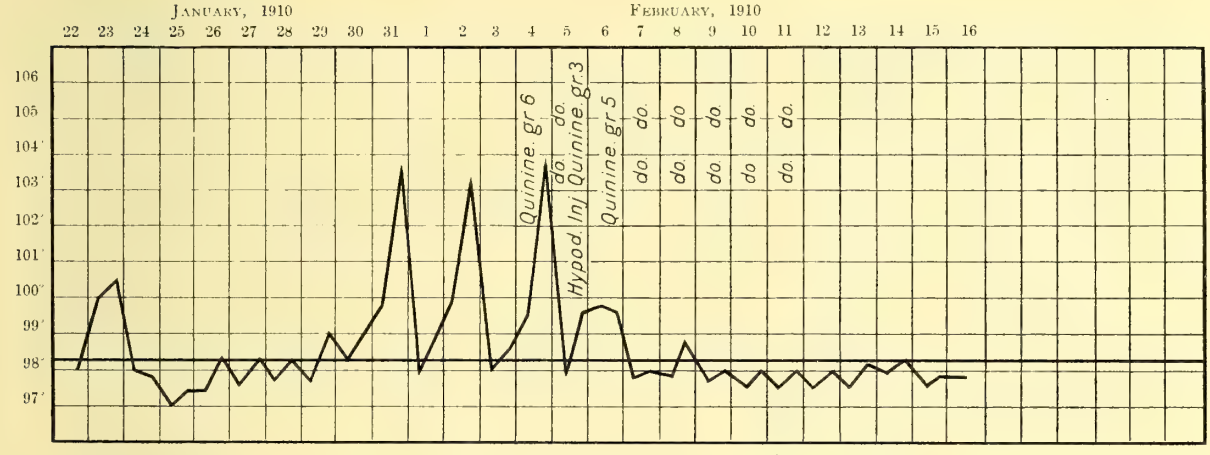


Fig. 68.—Chart 7, Acute Splenomegaly (Cause unknown)

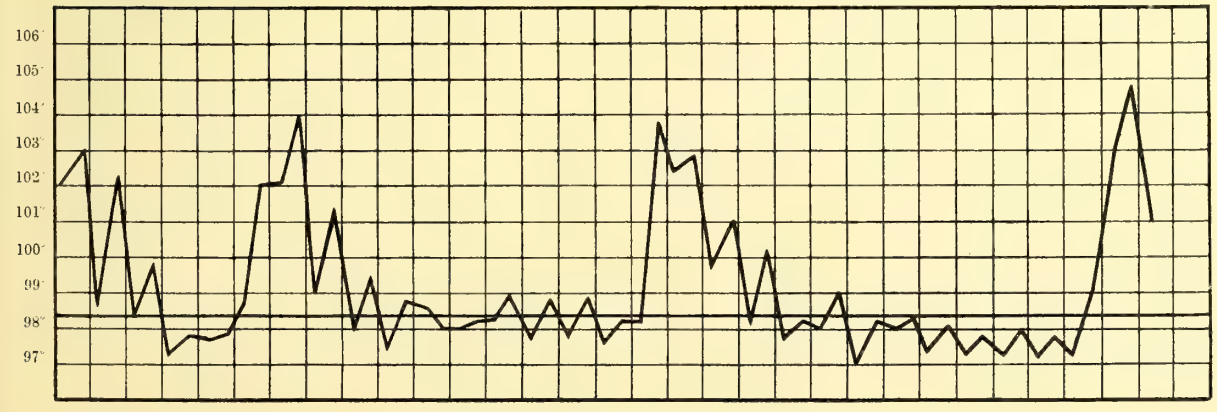


Fig. 69.—Chart 8, Pyelo-nephritis (?)

"*Present History.* For the last two months patient has had frequent attacks, every 4 or 5 days, of extremely severe pain in the left side accompanied by chill and high fever and severe vomiting; these attacks, instead of lasting one day as before, now last 2 or 3 days and are much more severe. They come on usually after 4 p.m.

History of a case

"*Physical Examination.* Patient much emaciated. *Heart* normal. *Lungs* normal. No enlargement of *liver* or *spleen*. *Alimentary Tract* normal. *Genito-Urinary Tract.* Tenderness over left kidney increasing during attacks, also slightly duller on percussion; no swelling; superficial veins of left side engorged. *Urine.* The urine is very peculiar in that it is not liquid like water, but it has the consistency of the white of an egg; if freshly examined it contains no precipitates, has a neutral reaction, and a low specific gravity, 1010–1015. The amount of urine increases during the attacks; it amounted to 5 pints in 24 hours during this last attack. During the attacks and for a day or two afterwards the urine contains albumin. I regret I have no microscope here and can have no microscopic examination of the urine made."

From reading this, one would think at once that the patient is suffering from attacks of pyelo-nephritis. At the same time a look at the temperature chart would suggest relapsing fever. I am personally inclined to think that the patient is suffering from inflammation of the kidney and its pelvis, and hope that the blood examination will throw more light on the subject. There is no history of syphilis or gonorrhœa or bilharzia.

On examining the blood film I found a distinct increase in the polymorphonuclears and noted the presence of a few myelocytes. Further search revealed a streptococcus. I only found one specimen in the two blood films sent, but it suggested a pathogenic form, and, considering the history, the other blood findings and the peculiar temperature, I believe the case to have been one of septicæmia, following some septic renal condition.*

One must, however, remember that mild cases of *B. coli* infection of the urinary tract are very apt to be overlooked and severe cases to be misunderstood. It is said that after children have been ill for some weeks with infection of this kind their blood sera in high dilutions will agglutinate the typhoid bacillus. (Porter and Fleischner.)

Despite all advances in blood culture, blood work, bacteriological investigations, increased knowledge in protozoology and helminthology, there still remain in the Sudan certain fevers of short duration which baffle the observer and are vaguely attributed to sun, chill, or fatigue. In some cases such a diagnosis may be correct. There is no reason for supposing that one or any of these agents may not upset the heat-regulating mechanism in such a way as to produce a temporary bout of fever, but one would not expect such fever to drag on from day to day—for say a week or longer. Here, for example, are some records and charts very kindly submitted by Major Standish O'Grady, R.A.M.C., the present S.M.O., British Troops in Khartoum, and well worthy of study.

Fevers of uncertain origin

Notes on Three Cases of Pyrexia of Uncertain Origin at Khartoum

These three cases, L. C. and W., were admitted to the Military Hospital, British Troops, on, respectively, September 30, October 18 and 24, 1910.

On admission. The appearance, symptoms and physical signs of each of these patients on admission were practically identical, and the course of the pyrexia also, as shown by the temperature charts, was very similar.

* Dr. Squires has since kindly informed me that the case turned out to be one of stone in the kidney.

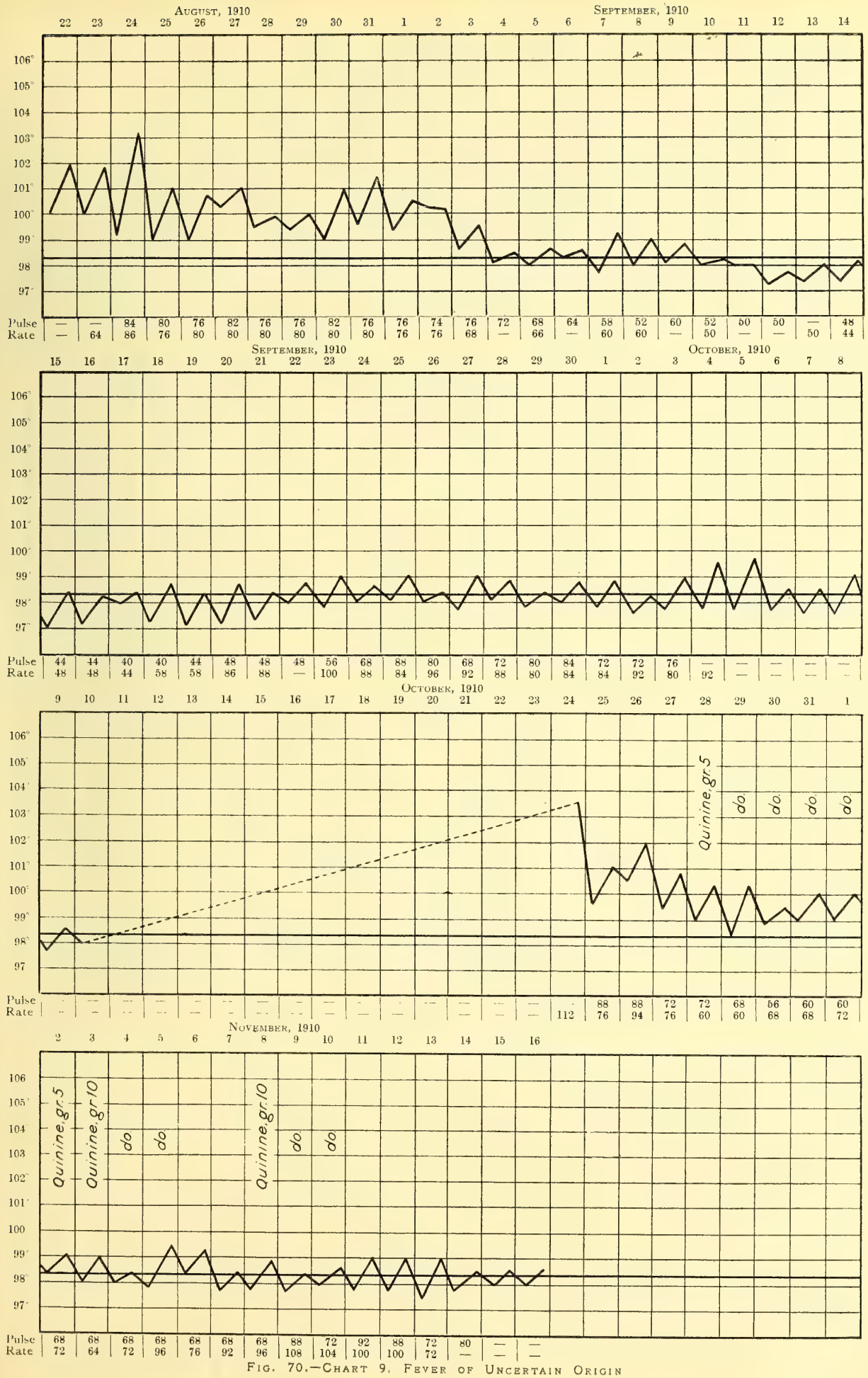


FIG. 70.—CHART 9. FEVER OF UNCERTAIN ORIGIN

Moves. C. (*Chart* 10) came straight to Khartoum from England. L. (*Chart* 9) and W. (*Chart* 11) had spent 2 years in Cairo, but had never had fever or been in hospital.

L. had an admission for a similar attack on August 22, 1910, as shown in Chart 9.

Previous History. C. was placed on the syphilis register on April 25, 1910, and is still undergoing a course of treatment by intramuscular injections of mercurial cream.

Symptoms. On admission the only symptoms and physical signs to be noted were those common to all cases of pyrexia, *i.e.* headache, with pains in the back and limbs; increased frequency of the heart beat, high-coloured urine with slight furring of the tongue. Several blood smears were made in each case which, though carefully gone through, showed no evidence of malarial infection. W. showed some increase of the large mononuclear leucocytes. None of the temperatures were affected by quinine.

Widal reactions with *B. typhosus* and *B. paratyphosus* gave negative results.

In the cases of L. and W. blood cultures were made using 5 c.c. of blood in each case; no growths were obtained.

There was nothing abnormal in the stools, nor in the urine.

Chart 12 shows the temperature record of a case in the Egyptian Military Hospital. The fever was of short duration. Malaria was excluded, quinine was not given and the nature of the disorder remained undiagnosed. Thanks to Captain Cummins I have similar charts of other cases where quinine was given, but without altering the temperature in the slightest.

Probable rôle of coliform organisms in etiology

Personally, I am inclined to put down some of these cases to the action of *B. coli* or allied organisms, the so-called paracoli bacilli which bear the same relation to the *B. coli* of Escherich as *B. paratyphosus* A. and B. to the *B. typhosus* of Eberth. There can be little doubt that one meets with cases ranging from a simple bowel upset of *B coli* origin through the paratyphoid group to the typical enteric fever. It has even been suggested that possibly the presence of ptomaines may so disturb the economy as to permit bacteria, carried in with the food or normally present in the intestines, to pass the ordinary barriers and invade the blood-stream, thus giving rise to a form of blood-poisoning.

In this connection Roger's short chapter on "Unclassified Long Fevers," is worthy of study. Whether one type can be transformed into another is a vexed question into which we need not enter, but as time goes on more links will undoubtedly be added to the imperfect coli-typhoid chain.* A step in this direction has been taken in these laboratories, for Captain Archibald has made the first serious attempt to separate out and classify these fevers. The work is tedious and far from complete, but that it is of practical value is, I think, shown by the results of the bacterial vaccine treatment he has tried in three cases as will be found recorded in his attached paper to which these brief notes serve merely as a general introduction.

* See papers in the *Journal Royal Army Medical Corps* for December, 1910.

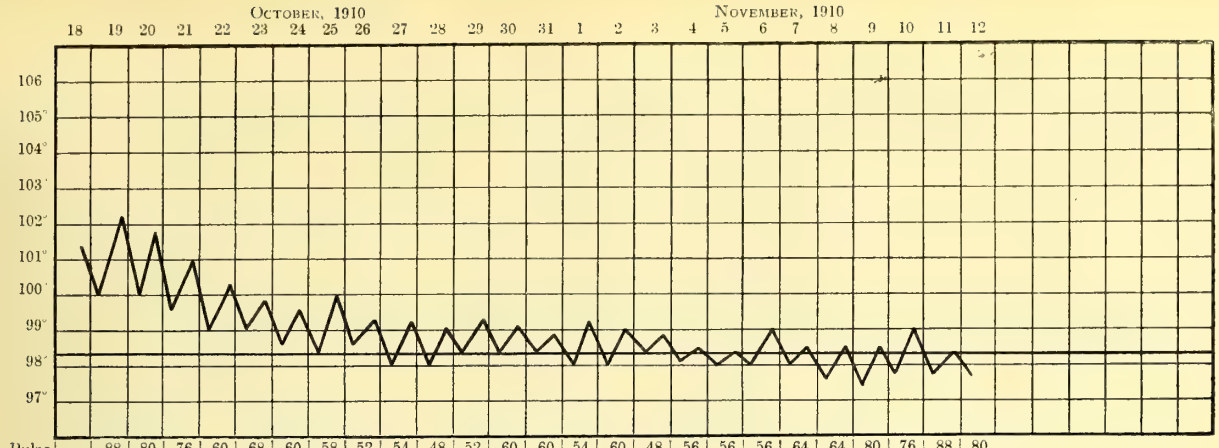
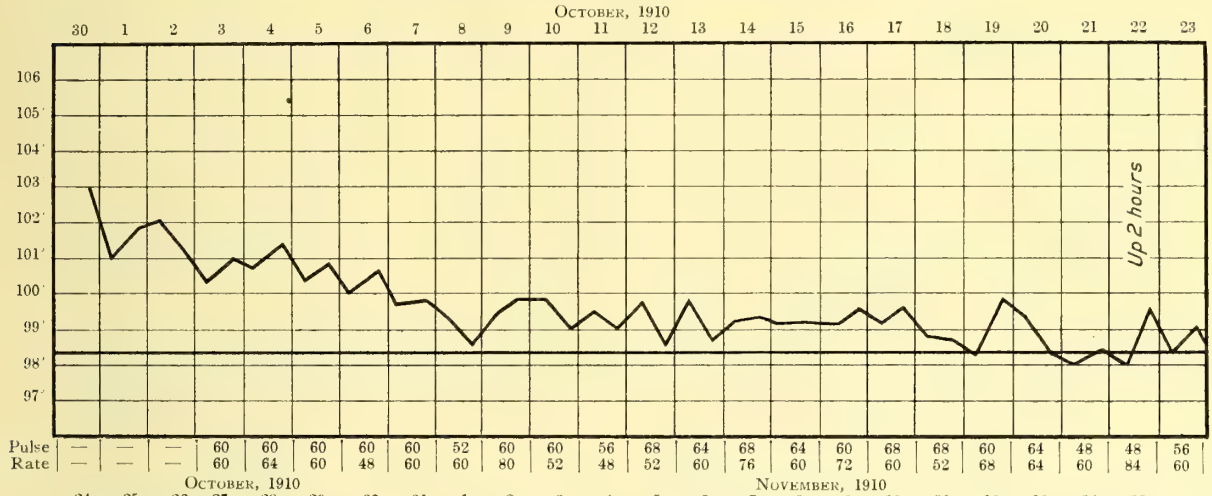


Fig. 71.—Chart 10, Fever of Uncertain Origin



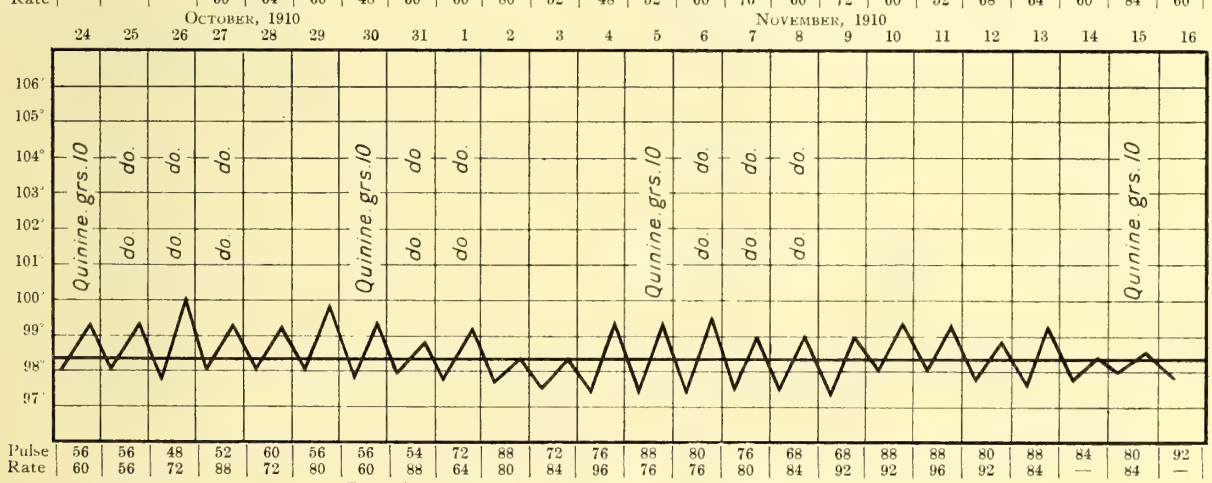


Fig. 72.—Chart 11, Fever of Uncertain Origin

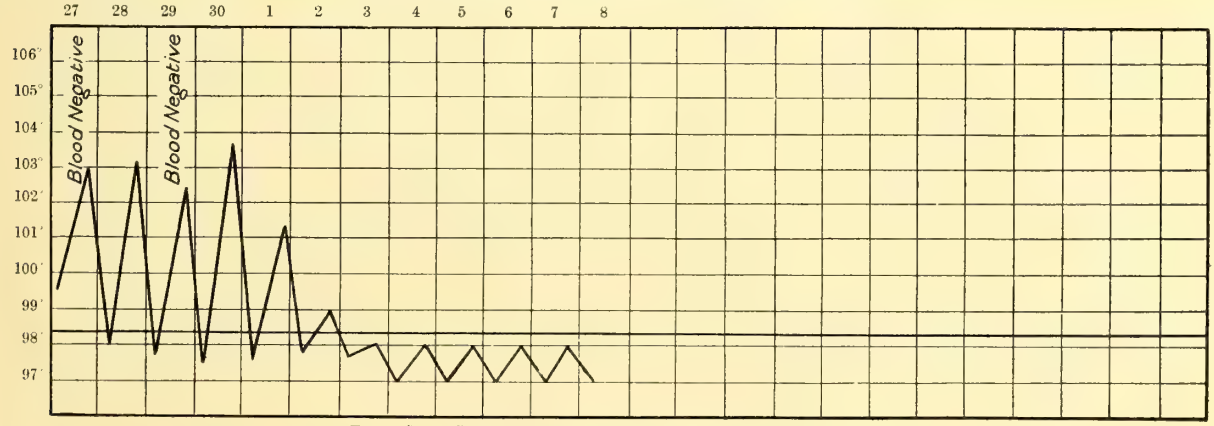


Fig. 73.—Chart 12, Fever of Uncertain Origin

Note.—In this Case no quinine was given. Compare with Chart 11 in which injections of quinine were given with no apparent effect on the temperature.

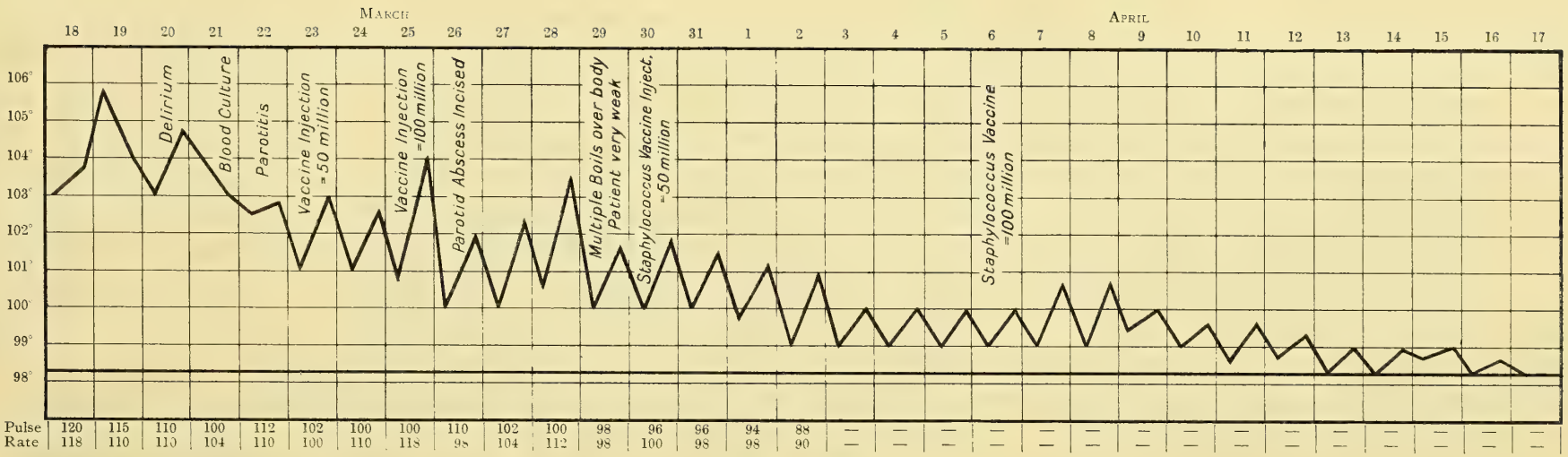


FIG. 74.—CASE 1

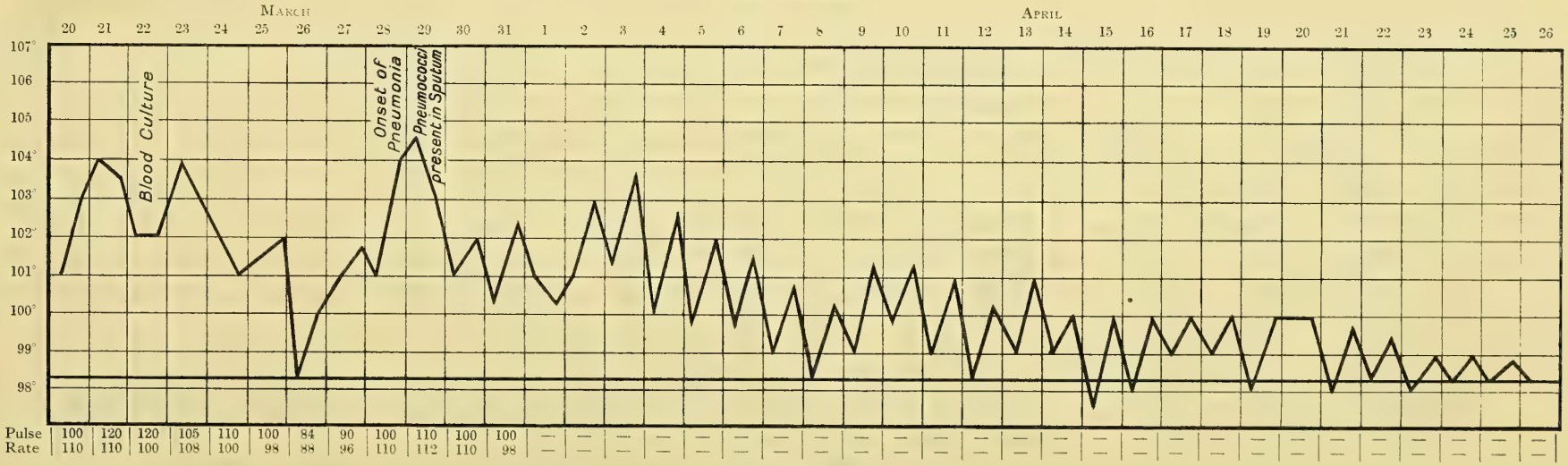


FIG. 75.—CASE 2

Four Cases of Pyrexia due to Infection with a Bacillus of the B. Cloacæ Type with Notes on the Vaccine Treatment of Two of these Cases

by

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The researches in tropical fevers have of recent years achieved such success, that it has now become possible not only to classify many of these fevers according to the type of pyrexia present, but also to state their etiology.

Many, however, of what constitute the so-called low fevers, pyrexias of uncertain origin, pseudo-typhoid, etc., require further light to be thrown on them to justify their present nomenclature, and one is inclined to think that, if a systematic series of blood cultures was carried out on all such types of fevers, their etiology would gradually be disclosed.

It is a common experience in these laboratories, more especially in the hot weather, to have a number of blood capsules sent from cases of pyrexia with the request that agglutination tests with the *B. typhosus, paratyphoid group,* and the *M. melitensis* be carried out. The results of these tests have, for the most part, been negative, as enteric and Malta fever are not common in Khartoum. Examinations of the blood films of these cases eliminate malaria, and the question of a tubercle infection is then considered. Eventually, in the course of ten days or so, recovery occurs, and, as a rule, no definite diagnosis is arrived at.

Blood culture for early diagnosis

It is for such cases of pyrexia that one puts in a plea for the procedure of blood culture, a procedure not to be carried out as a *dernier resort* after agglutination tests, etc., have failed, but to be employed in the early stages of the disease.

During the summer of last year, various types of fevers presented themselves, and amongst the most interesting were three cases, which occurred in the 3rd Egyptian Battalion, and one case which occurred in an Englishman. These four cases were of interest, in that they resembled each other clinically, and in three of them the same micro-organism was isolated by means of blood culture.

The three cases from the Egyptian battalion were all admitted to the Khartoum Military Hospital within a few days of each other, while the fourth case occurred somewhat later. Through the kindness of Captain A. G. Cummins, R.A.M.C., one was able to receive full notes, together with the temperature charts, of these cases. I am also indebted to that officer for the great assistance rendered in carrying out the blood cultures, etc. For the notes and temperature charts of Case IV., which was admitted to the Khartoum Civil Hospital, I am indebted to Dr. H. C. Squires, of the Sudan Medical Department.

For convenience, the cases in the Egyptian Battalion will be named Cases I., II. and III., while that occurring in the Englishman will be described as Case IV.

In Cases I. and IV. a vaccine treatment was employed, and they will be described in fuller detail.

The fever presented the following characteristics :—

Clinical signs

Severe headache was usually a marked symptom on admission, associated more or less with drowsiness and sometimes delirium. The tongue was furred and dry, and the pulse was usually rapid. The initial pyrexia was a high one, running between 101° F. and 103° F. No rash was present, and only in Case IV. did the spleen show any evidence of being distinctly enlarged. In Cases I., II. and III. the spleen was just palpable and somewhat tender. Diarrhœa was not a marked symptom, nor was tympanites present. The clinical appearance of the patients was very suggestive of enteric in the second week. A noticeable feature was the tendency of the temperature to become normal at the end of the first week, to be invariably followed by a remittent type of pyrexia which lasted a variable period. Complications presented themselves in three out of the four cases. Case I. developed parotitis on the fourth day, followed on the tenth day of the disease by an outbreak of multiple boils. Case II. developed pneumonia on the seventh day, while Case III. showed signs and symptoms of femoral thrombosis on the eighth day. Blood serum was taken on several occasions from all four cases and subjected to agglutination tests with strains of *B. typhosus*, *B. paratyphosus*, and *B. dysenteriæ* Shiga, but all with negative results. These agglutination tests were undertaken at the end of the fever as well as during the first week.

In Cases I. and II. blood cultures were carried out on the fourth and third days of the disease respectively, while in Case IV. the blood culture was not undertaken till the fifth week. As previously stated, the same micro-organism was present in the blood of these three cases. Owing to pressure of work, no blood culture was carried out in Case III., but the serum reaction of this patient was tested with the micro-organisms found in the blood of Cases I., II. and IV., and a positive agglutination was obtained in a dilution of 1 : 100. It will now be convenient to note the characters of this organism.

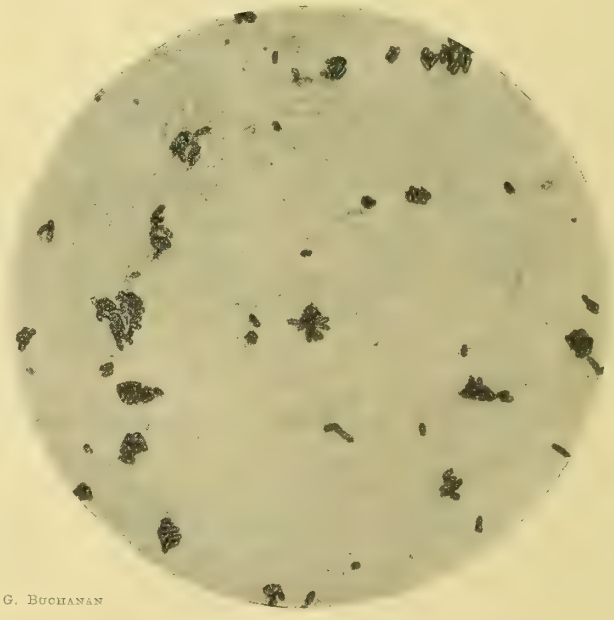


Fig. 76.—Bacillus from Case IV.

Isolation of the organism

In carrying out the blood culture, the usual careful precautions for sterilising the skin over the median basilic vein were observed. By means of a sterile hypodermic syringe 5–10 c.c. of the patient's blood were withdrawn, and flasks containing Coleman's[1] and Bunton's bile glycerine medium were inoculated, as well as flasks containing sterile broth. These flasks were then incubated at 37° C. for 48 hours, and examined for the presence of micro-organisms. Samples from all the flasks were then plated out on Drigalski-Conradi agar, bile-salt neutral-red agar, and ordinary agar, the colonies picked off, and various culture media inoculated in order to study more in detail the characters of the organisms isolated. These were as follows :

On Drigalski-Conradi, at the end of 48 hours, there were small circular translucent pearl-like colonies present. By transmitted light such colonies appeared to have a small

[1] Coleman, H., and Bunton (June, 1907), *American Journal of Medical Science.* Quoted in *Epit. British Medical Journal*, April 25, 1908, p. 68.

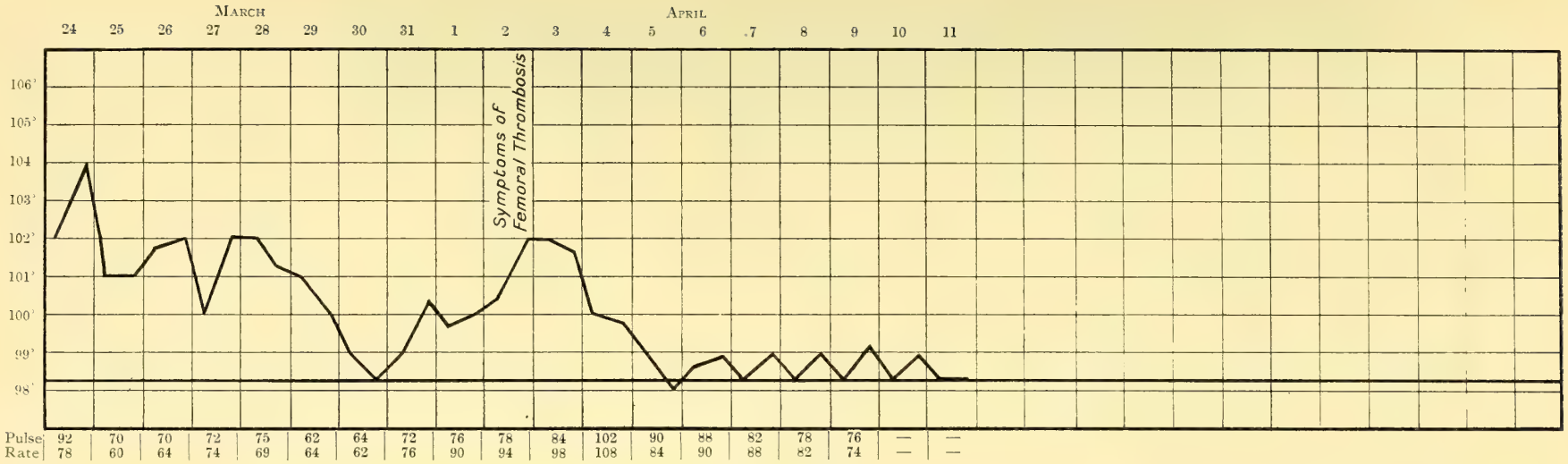


FIG. 77.—CASE 3

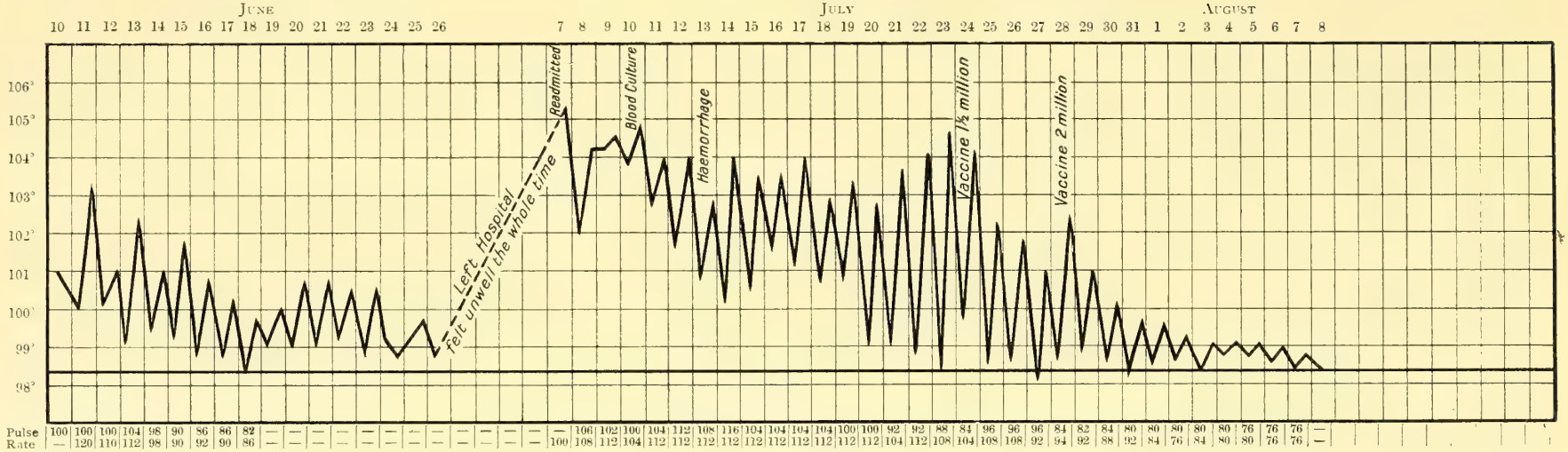


FIG. 78.—CASE 4

opaque centre surrounded by a translucent rim. A similar appearance was produced on Endo's fuchsin-agar. Acid was not produced in either of these media. On Fawcus's medium the growth appeared as minute translucent dew-drop like colonies resembling somewhat in appearance those of *B. typhosus*. Agar slopes inoculated with this organism showed a greyish-white film with irregular margins. At the end of 24 hours the growth in this medium was not a luxuriant one. In broth the organism produced a cloudiness without the formation of a pellicle.

Hanging-drop preparations taken from a young culture showed the presence of a very small, actively motile organism of the nature of a cocco-bacillus. Stained preparations showed a cocco-bacillus measuring on an average 1·6μ in length. Some of the larger forms measured 1·8μ. In agar cultures the organism assumed more the type of a small bacillus, the coccoid forms having disappeared. It was easily stained by most of the basic aniline dyes, was Gram-negative, non-acid fast and non-spore forming. Its optimum temperature was 37° C., while its minimum and maximum temperatures were 32° C. and 52° C. respectively. It was a facultative anærobe. On blood serum it produced a yellow creamy growth, but no digestion occurred, while on potato, minute translucent colonies appeared at the end of 48 hours. At the end of a week these were somewhat buff-coloured.

Morphology

It was non-pathogenic to guinea-pigs and rabbits. In the latter a distinct rise of temperature occurred and lasted for 4 days. The specific sera of *typhoid*, *paratyphoid A.* and *paratyphoid B.* failed to agglutinate it.

The following reactions occurred in the various sugars, etc.:—

Lactose	Glucose	Saccharose	Dulcit	Adonit	Inulin	Mannite	Maltose	Litmus Milk	Litmus Whey	Levulose	Galactose	Raffinose	Starch	Indol	Voges and Proskauer Reaction	Glucose Bile-Salt Neutral Red
—	+	—	+	—	—	+	+	A. ALK.	A.	+	+	—	+	+	+	Gas, but no fluorescence

+ = Acid and gas. — = No acid and no gas.
A. = Acid only. ALK. = Alkalinity.

Cultural characters

In litmus milk there was slight acidity after three days. At the end of ten days, no clotting had occurred, but the litmus was totally discharged, the milk remaining white in colour. At the end of a month the reaction of the medium was alkaline.

Gelatine as a culture medium could not be employed owing to the existing climatic conditions.

Although the cultural reactions of this organism did not correspond with those of the *B. typhosus* and the *paratyphoid group*, the fact that it might have been a modified variety of them justified further differentiation tests being carried out. Three rabbits were immunised by injections of killed cultures of *B. typhosus*, *B. paratyphosus B.* and the recently isolated organism.

A series of agglutination tests was then carried out between the sera of the animals immunised against *B. typhosus* and *B. paratyphosus B.* and the new organism in question. The agglutination tests were made by the macroscopic method. Instead of broth cultures, emulsions made in normal saline solution, from agar cultures grown at 37° C. for 24 hours,

were employed, and in order that the emulsion of the various bacilli should in each experiment be approximately of the same opalescence, a series of tubes of the same calibre was used and a paper, having parallel lines on it, placed behind these tubes. The strength of the emulsion was gauged when these parallel lines were just visible through the tubes containing the emulsion. A reading of the agglutination was taken after the tubes had been incubated at 37° C. for two hours, and a second reading was taken 24 hours afterwards. These macroscopical findings were also checked microscopically, and the highest agglutinating titre was thus estimated, and was as follows:—

Agglutination tests

B. typhosus rabbit's serum, agglutinated a strain of *B. typhosus* in a dilution of 1 in 100 in 24 hours.

B. paratyphosus B. rabbit's serum, agglutinated *B. paratyphoid* in a dilution of 1 in 400.

The serum of the rabbit, inoculated with the new organism, agglutinated this organism in 1 in 200 in 24 hours. This serum was then tested against the strains of *B. typhosus* and *B. paratyphosus B.*, and the results of this experiment were as follows:—

Time limit 2 hours

Serum	Dilution	Agglutination limits		
		1. New organism	2. *B. typhosus*	3. *B. paratyphoid*
Serum of rabbit inoculated with new organism	1— 20	Complete	Complete	Partial
	1—100	ditto	Nil	Nil
	1—200	ditto	Nil	Nil

Time limit 24 hours

Serum	Dilution	Agglutination limits		
		1. New organism	2. *B. typhosus*	3. *B. paratyphoid*
Serum of rabbit inoculated with new organism	1—100	Complete	Nil	Partial
	1—200	ditto	Nil	Nil
	1—400	ditto	Nil	Nil

In order to employ further differentiation tests between this new organism and the *B. typhosus* and *B. paratyphoid B.*, a few absorption tests were carried out. The method employed was that used by Castellani[1] in order to differentiate organisms further than by their agglutination reactions.

The principle of the test has been really applied as a diagnostic aid in cases of mixed infections, and, as an aid to differentiating allied groups of organisms, it ought to be a valuable one, for it stands to reason that, if specific agglutinins can be produced in a serum as a result of vaccination with a known organism, and if these agglutinins can be removed by this organism as well as by a heterologous one, when the serum is saturated with them, then these two organisms are probably identical. Bainbridge[2] has applied this principle in a series of absorption tests carried out with food-poisoning bacilli and found it a reliable one.

Absorption tests

[1] Castellani, A. (1902), *Ztschf. f. Hyg.*, Leipzig, Bd. XL., S. 1.

[2] Bainbridge, F. A. (April, 1909), "Paratyphoid and Food-poisoning Bacilli." *Journal of Pathology and Bacteriology.*

In carrying out these tests the organisms that were used for the absorption of the agglutinins were obtained from agar cultures twenty-four hours old. The dilution of the serum used was 1 : 20.

The technique was as follows:—The serum of the rabbit immunised against the new organism was saturated for 3 hours at 37° C. with a 24-hours' culture of this organism. The serum was then centrifuged and the clear supernatant fluid withdrawn and mixed in dilutions of 1 : 20 with (*a*) the new organism (*b*) *B. typhosus* (*c*) *B. paratyphoid B.*, and the results observed were as follows:—

In (*a*) mixture no agglutination occurred as all the agglutinins for this organism had been previously absorbed when the serum was saturated with it. In (*b*) and (*c*) agglutination occurred.

Pressure of work prevented further research being carried out to differentiate this organism, and, had it been possible to perform them, absorption tests with other allied ones would doubtless have assisted one in coming to a more definite conclusion as to its type.

From the cultural reactions obtained, there can be no doubt that it is closely allied to the *coli* group, but its reactions do not appear to correspond with any identified organism.

Cultural relationships

The fact that it gives a positive Voges-Proskauer reaction, rather points to its belonging either to the *B. cloacæ* or *B. lactis ærogenes* group. The latter group may be excluded as all bacilli belonging to it are lactose fermenters.

MacConkey,[1] in his carefully worked out investigations on the lactose fermenters in fæces, has looked for the Voges-Proskauer reaction in a large number of organisms but only found it when organisms of the *B. lactis ærogenes* or *B. cloacæ* group were present.

In the *B. cloacæ* group, Ford[2] described four different varieties and, according to that observer, they are not all lactose fermenters.

B. cloacæ is an organism frequently found in the fæces of cattle, but its common habitat is the soil. Clemesha,[3] in India, found it frequently in the beds of lakes and rivers, and it has a greater resisting power against the action of sunlight than all other coli types with the exception of *B. coscoroba.*

Presence of the organism in the excreta

Attempts were also made to recover the organism from the urine and fæces of these cases. In Case I. and Case IV. it was found to be present in the fæces in the second and fifth weeks of the disease, respectively. No evidence of it was found in either the fæces or urine of Cases II. and III., nor was it present in the urine of Case I. Unfortunately, no attempts were made to isolate it in the urine of Case IV.

The method of infection in these cases was, in all probability, due to food or water contamination. The three cases occurring in the same battalion were probably infected from the same source. Case IV. occurred in an Englishman living in Khartoum North, where the water-supply is frequently obtained from wells.

To sum up the leading points:—

1. There exists in Khartoum an irregular remittent fever which is due to a systemic infection with a coli type of organism akin to the *B. cloacæ* group.

2. The fever is a characteristic one, commencing with high initial pyrexia lasting for three or four days, falling to normal on the seventh or eighth day, after which a relapse occurs and an irregular remittent fever is produced, lasting for an indefinite period and liable to be associated with complications.

[1] MacConkey, A. (July, 1905), "Lactose fermenting bacteria in Fæces." *Journal of Hygiene.*

[2] Ford, W. W. (1901), *Journal of Medical Research*, Vol. VI., p. 211.

[3] Clemesha, Wm. (1909), "A study on the bacteriology of drinking-water supplies in Tropical Climates." *Appendix to the Annual Report of the King Institute of Preventive Medicine*, Madras, for 1908.

3. The signs and symptoms simulate somewhat a case of enteric in the second week of the disease.

4. The fever is a septicæmia and the bacillus has been isolated from the blood as late as the fifth week of the disease. The blood serum has no agglutinative reaction on *B. typhosus* and *B. paratyphoid B.* and *B. dysenteriæ*, but agglutinates the specific bacillus up to a dilution of 1 : 100.

5. In two of the cases the bacillus was isolated from the fæces.

Notes on the Treatment of two of the Cases by means of Vaccines

It is the usual custom in these laboratories to prepare a vaccine of any organism isolated from the blood of cases of severe pyrexia, occurring in Khartoum. Treatment with such a vaccine is shown to be justifiable if one refers to the account of two of these febrile cases treated by autogenous vaccines. The most marked result of vaccine treatment was obtained in Case IV., that of the Englishman. Case I. was of interest owing to the fact that the patient received two different vaccines. For the notes on this case I am indebted to Captain Cummins, R.A.M.C. The following were the chief points. Four days after admission a blood culture was carried out and a vaccine made of the above-mentioned bacillus. The patient's general condition was serious, passing from a state of delirium into one of coma. The pulse was weak and rapid. There was some enlargement of the parotid gland. An injection of ·5 c.c. of the vaccine representing 50,000,000 organisms was given on the sixth day of the disease.

For the next two days there was no change in the patient's condition. The swelling of the parotid gland had increased in size. A second injection of the vaccine was given on the eighth day, the amount used being 1 c.c. which represented 100,000,000 organisms. This was followed by a rise of temperature. The following day there was some improvement noted as the patient appeared more rational. An incision was made into the parotid swelling from which pus freely exuded. Two days later the trunk, arms and legs of the patient were covered by multiple boils, several of which were suppurating. There was also a purulent discharge from the right ear.

Outcrop of boils

He was now in a somewhat asthenic state, due doubtless to septic absorption. Many of the boils had broken down and formed large bed sores.

Cultures made from the pus present in these boils showed the presence of a *Staphylococcus albus*. A vaccine was made and an injection representing 50 million killed staphylococci was given subcutaneously. Fresh boils appeared on the scrotum. The temperature at this stage was showing an evening rise to 100° F. and a morning fall to 99° F. A second injection of the staphylococcus vaccine was given eight days after the original one, the dose representing 100,000,000 organisms. This was followed by such beneficial results that at the end of a week the boils had entirely healed. From this onwards, progress towards recovery was gradual but well maintained, and in three weeks the patient left hospital in a convalescent state.

Success of vaccine treatment

The history of Case IV. is of equal interest. For the notes on it, I am extremely indebted to Dr. Squires of the Sudan Medical Department.

Patient was admitted to hospital suffering from severe headache, malaise and pyrexia. On the second night after admission the temperature reached 103° F. and then fell by lysis till the eighth day, when a secondary rise occurred. After patient had been under

treatment for a fortnight he expressed a wish to leave hospital. He was re-admitted in a delirious state twelve days later with a temperature of 105·4° F. Malarial parasites were not present in the blood. Blood culture was carried out three days later, and a vaccine of the organisms isolated was prepared. Patient's condition steadily became worse. About a week afterwards he passed some bright red blood per rectum, and this was accounted for by the fact that there was a local surface abrasion in the vicinity of the anus. The condition of the patient at this stage was somewhat critical. The morning and evening temperatures showed very wide variations. Owing to his weak condition it was decided that only small doses of the prepared vaccine ought to be administered, and, accordingly, one and a half million of killed organisms was injected. Within 48 hours the temperature reached the lowest recorded since the patient's re-admission to hospital. The temperature then began to fall by lysis, and four days afterwards a second injection of the vaccine containing two millions of the killed organisms was given. This was followed by a slight rise of temperature, but the latter gradually fell by lysis, and in ten days had reached normal. The patient made an uninterrupted recovery, and left hospital soon after in a convalescent state.

Minimal dose of vaccine brings about great improvement

Dr. Squires stated that the improvement in the patient's general condition following the injection of the vaccine was the most striking feature of the whole case, and that there was not the slightest doubt that the dose, small though it was, had been sufficient to turn the scale in the patient's favour, although nothing in the way of a crisis was produced.

Conclusions

From the results obtained by the injections of autogenous vaccines in these two patients there can be but little doubt as to the efficacy of vaccine-therapy in such cases of septicæmic fever. The successful issue was due to the fact that autogenous vaccines were employed. The extra trouble necessitated in making autogenous vaccines is well repaid in the end by the results achieved, and that has been the experience of the writer, more especially in the vaccine treatment of that somewhat troublesome and resistant condition known as the Nile boil. The question of strength and dosage of the vaccines used is one that cannot be laid down by rule-of-thumb, as the chief guide to these points is to be found in the clinical condition of the patient. Further, in the writer's experience the opsonic index is totally valueless when compared with the clinical progress of the disease as a guide to the frequency of administration of vaccines.

Conclusions

In Case I., in all probability, too strong a vaccine was employed to produce immediate amelioration of the patient's condition, and one is inclined to agree with the remarks made by Captain Kennedy[1] in a recent paper that in all cases of acute infection the best results are obtained by the administration of a series of small doses of vaccine employed at short intervals. As a further plea in favour of employing autogenous vaccines in fevers of the septicæmic type the procedure involves the withdrawal of at least 5–10 c.c. of blood from a vein. Such a procedure has invariably a beneficial effect on the patient, and whether it is used as a means of diagnosis or as a method of isolating an organism for vaccine preparation, its practice should be more frequently encouraged.

It is of interest to note that Statham[2] in South Africa has isolated organisms of the *B. cloacæ* type from the blood of patients suffering from a typhoid-like pyrexia.

[1] Kennedy, J. C. (1910), "Vaccine Treatment of Malta Fever." *Journal of Royal Army Medical Corps.*, September.

[2] Statham, J. C. B. (December, 1910), "A Small Outbreak of Fever due to Bacteria having Unusual Cultural Characters." *Ibid.*

Diphtheria in the Tropics

Is there a coccal form of the *Bacillus diphtheriæ?*

BY

The Director

It is Rogers[1] who says "Diphtheria is fortunately much less common in the tropical East than in temperate parts of Europe and America. It does occur in Calcutta, and also in China, being also much more frequent in the cold season and especially rare in the rains." Doubtless it is this rarity which is the cause of the paucity of literature on diphtheria in tropical countries. Curiously enough, however, a paper has appeared in the *Journal of Tropical Medicine and Hygiene* for November 1, 1910, plaintively asking why more is not heard about diphtheria in tropical countries. In the Review Supplement to our Third Report I wrote: "In my experience, cases of diphtheria, so far as Khartoum is concerned, are apt to crop up in October, when the summer has been dry. I have never seen an extensive epidemic nor have I ever been able to trace the disease to its source. Apparently it has been introduced from outside, and it is usually of a severe form associated with streptococcal infection. The type of diphtheria bacillus present has, as a rule, been what Westbrook would term Granular C. The disease is undoubtedly rare in the Sudan." Diphtheria in the Sudan

I have looked through Ruge's[2] exhaustive review of diseases in the Tropics in Virchow's *Jahresbericht* for the last three years and find scarcely any reference to diphtheria, while in my own references the only paper worth mentioning is one by Castellani,[3] who discusses the subject much more fully than Rogers, describes a typical severe case in Ceylon, and concludes as follows:—

"This case, together with the previous observations of Perry, Fernando and da Silva in Ceylon, and Plehn, Cornwall, etc., in other parts of the Tropics, tends to demonstrate that diphtheria is not limited simply to the temperate and sub-tropical zones, but that it is a true cosmopolitan disease, as it occurs also, and probably much more frequently than hitherto supposed, in tropical countries. The different climatic conditions may, perhaps, influence in some instances the characteristic clinical features and course of the disease, and this, if a bacteriological examination is not taken (*sic*), may render difficult the diagnosis, and lead to some cases being overlooked."

The disease would, however, appear scarcely to merit particular attention but for the fact that on several occasions I have encountered in undoubted diphtheria cases what I believe to be a coccal form of the Klebs-Lœffler bacillus. The only references to any similar finding which I can discover are in Park's *Pathogenic Micro-organisms*,[4] where an illustration is given of segmented granular coccus forms grown on Lœffler's serum, and obtained from cases of diphtheria at a children's home in New York, and in a paper by Trautmann and Dale[5] with a sequel by Dale[6] alone on coccoid forms found in culture during the course of an epidemic in Hamburg. A coccal form of the Diphtheria bacillus

[1] Rogers, L. (1910), *Fevers in the Tropics*. 2nd Ed., London.

[2] Ruge, S. (1907–8–9), "Tropenkrankheiten." Virchow's *Jahresbericht der gesammten Medicin*, Band I.

[3] Castellani, A. (May 2, 1904), "Diphtheria in the Tropics." *Journal Tropical Medicine*, Vol. VII.

[4] Park, W. H. (1908), *Pathogenic Micro-organisms*. 3rd Ed., London.

[5] Trautmann, and Dale, J. (1910), "Beitrag zum Formenkreis des Diphtheriebacillus." *Cent. f. Bakt.; Beilage zu Abt.* 7, Ref. Vol. 47, p. 203.

[6] Dale, J. (December 7, 1910), "Ueber eine ungewöhnliche Form des Diphtheriebacillus." *Cent. f. Bakt.;* Orig. Vol. 56, Nos. 5/6.

I have gone through the laboratory records from July, 1903, to November, 1910, and, during this period, I find only 24 cases of diphtheria have been diagnosed bacteriologically. Of these, swabs from nine came from Khartoum, one being certainly a case in which infection took place in Egypt or on the way to Khartoum. In ten cases the swabs were sent from Omdurman, while Atbara (2), Shendi (1), and Dongola (1), supplied the remainder. There were only 4 cases in Europeans, one of which was imported.

No less than 15 of the 24 examinations were made in September, October or November, the greatest number being in October. There were three in December, two in January, two in March, one in February and one in August. It is possible that this greater tendency for cases to occur in the autumn is due to the influx of persons from Egypt at this season. No doubt carrier cases are introduced but, as a rule, the disease does not spread, possibly on account of the dryness of the atmosphere. That it may become epidemic in the Sudan is shown by an outbreak which occurred early in January, 1910, amongst Arabs in certain villages not far from the Nile in the Upper Nile Province, and which caused 19 deaths out of a total of 26 attacked. The natives themselves recognised the disease which they call "Luhana" and which was laryngeal in type, as mentioned by the Medical Officer in charge of Dueim, whose interesting report I have seen, thanks to the courtesy of Colonel H. B. Mathias.

Seasonal incidence

The climate in these more southerly regions is of course more humid, and there were stagnant water pools in the neighbourhood of the villages scourged by the epidemic.

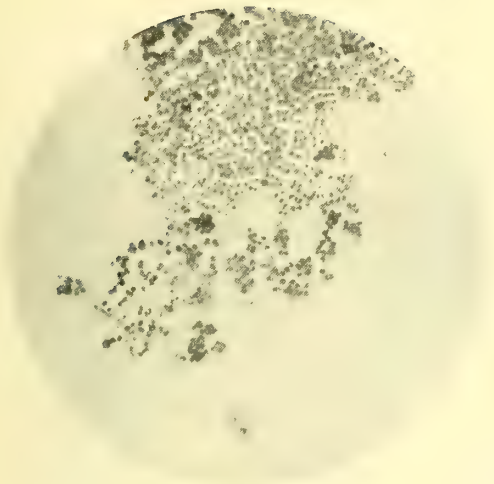
Of the 24 cases mentioned, three are described as "coccal," and a fourth, a contact case, as doubtfully coccal. One has to distinguish between cases which show coccal forms in swabs from the fauces and those in which coccal forms appear in culture on agar or blood serum. What first drew my attention to the subject was the finding of what was practically a pure culture of curious cocci in films made from a swab sent from a case at Atbara *which, clinically, was typical diphtheria and which proved fatal.* These cocci showed Neisser's granules when stained by his method and gave characteristic *B. diphtheriæ* colonies on Lœffler's serum. Unfortunately my specimens of this case were lost in the fire, and, though I met with one or two other coccal cases, it was not until late in 1909 that I had a chance of making any detailed study of the condition. At that time we had some diphtheritic material in the laboratory from a case of considerable interest (*vide infra*) which had occurred in Khartoum.

A Khartoum case

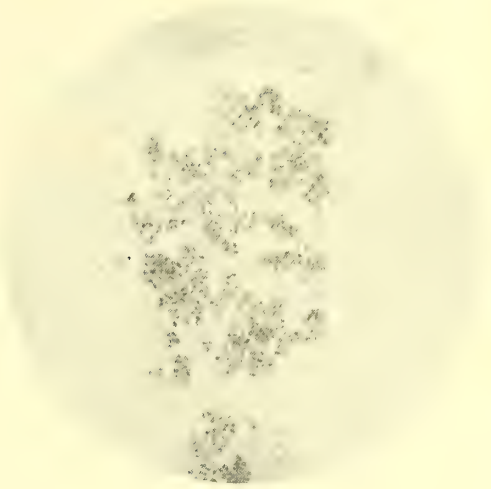
On December 10 one of our native laboratory attendants who, amongst other things, had been sterilising old cultures, complained of a sore throat. I examined him, found patches of membrane in his fauces and discovered enlarged and painful glands beneath his lower jaw angles. He had no temperature. His throat was swabbed and typical *B. diphtheriæ,* together with a mixed flora, found in films made direct from the swab. The swab was rubbed over a slant of Lœffler's serum. Colonies all of one type, morphologically like those of *B. diphtheriæ,* but microscopically composed wholly of Gram-negative cocci appeared within 24 hours (Incubation at 37° C.). Either because the significance of this result was not at the moment recognised, or for some other reason which I have forgotten, these coccal cultures were not followed up.

The man was isolated as a very mild "contact" case and, the supply of antitoxin being limited, was given formamint in the form of tablets. He sucked these with such vigour that his throat was clear on the following day and his glands had improved, but, on December 15, a yellow, follicular plug was seen on the right tonsil. On swabbing, this yielded only Gram-negative diplococci, to all appearance identical with those which developed on the serum cultures made on December 10. They contained granules which

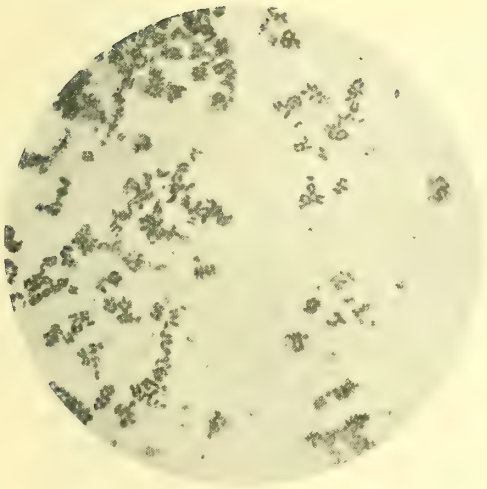
PLATE XIV



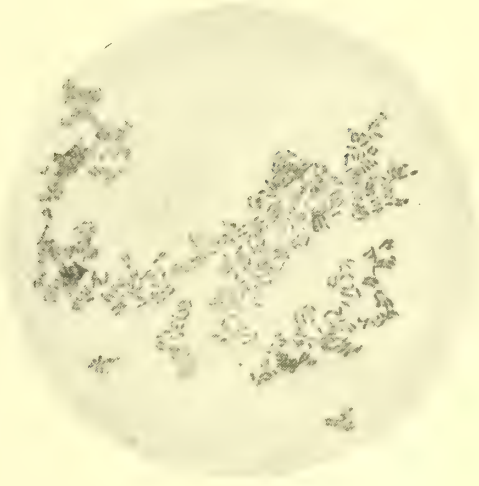
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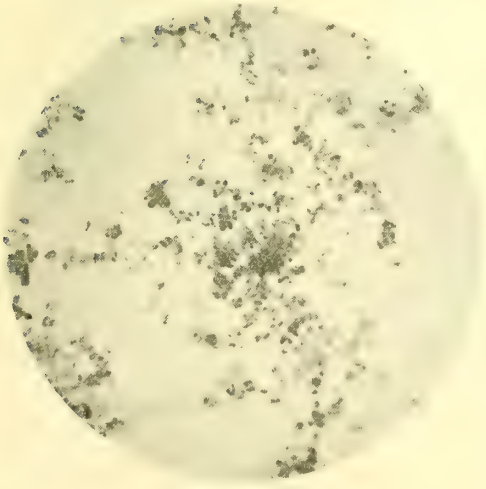
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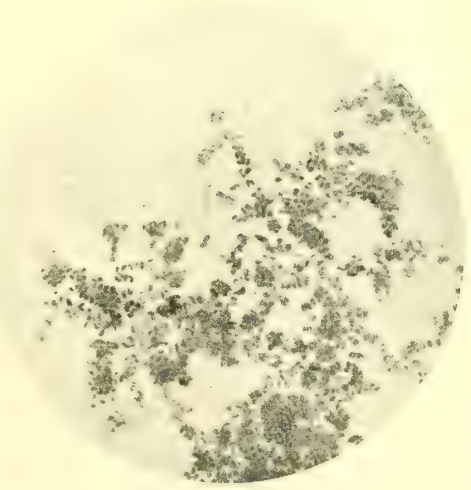
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6

G. Buchanan

DIPHTHERIA

1. Coccal form showing metachromatic granules.
2. Typical beaded rods.
3. Change from coccal to rod form.
4. Change more pronounced. Some rods show polar bodies.
5. Old culture. Barred involution forms.
6. Still older culture with swollen and involution forms.

× 1000 *diam.*

reacted faintly to the Neisser stain (Plate XIV., fig. 1). A pure culture of these cocci was obtained on serum and on agar, both in tube and on plates, and it was felt that the case was worthy of extended investigation, especially as the man's throat continued to yield these cocci for some time, although he became to all appearance perfectly well. Owing to the pressure of other work it is unfortunate that the exact date on which his throat was found free of these cocci was not noted, while it is a pity that he was not given a single dose of antitoxin and its effect on the micro-organisms observed.

Bacillary form predominates on subculture on agar

However, it was soon found that on subculture on agar, *i.e.* within 48 hours, the cocci assumed a bacillary form, so that at first there were coccal and bacillary forms together, and then, in some cases, bacilli alone (Plate XIV., figs. 3 and 4), all the cocci having assumed the rod form. Attempts to separate the coccal from the bacillary forms by means of agar and serum plates entirely failed. They were evidently the same organism. Every care was taken in the culture work. My assistant, Mr. Buchanan, worked with one set of plates while I worked with another, and our results were identical. The reaction of the agar used was always +10 on Eyre's scale, except on certain occasions *(vide infra)*. The bacilli, as they developed, proved to be both Gram-positive and Gram-negative and to resemble *B. diphtheriæ* morphologically, especially when transferred to broth. They showed metachromatic granules when stained with Neisser. Cultures were incubated at 37° C. and then kept at winter room temperature (about 95° F.) exposed to the light. On agar slope, examination with a magnifying lens showed the small, crowded, white colonies to be discrete.

It was found that original coccal cultures on blood serum slopes remained coccal. The colonies were small and discrete, and the cocci, on staining with Gram, proved negative save for granules which, in a certain proportion of them, retained the stain. Cultures on serum plates, kept at the room temperature, eventually showed both forms. The agar cultures and subcultures showed the progressive change of cocci into bacilli. The coccal form appeared to divide and produce a small diplo-bacillus and this gradually became the apparently typical *B. diphtheriæ*, showing polar bodies when stained by Neisser's method, barred forms and forms like those shown in Plate IX., fig. 2, of Nuttall's and Graham-Smith's standard work.[1] Involution forms exactly resembling those of the Klebs-Lœffler rods eventually developed, and, in old agar cultures, the bacilli tended to become Gram-negative. Naturally it was not sufficient to base any conclusions on morphology alone, and the following cultural reactions were determined.

Involution forms

1. Ordinary alkaline broth. The growth exactly resembled that of *B. diphtheriæ* in this medium, there being no turbidity but a formation of whitish granules at the bottom and along the sides of the tubes. In older subcultures a slight film formed on the surface which, on shaking, broke up and sank slowly to the bottom of the broth.
2. Glucose broth. In 1 per cent. glucose broth the organism, whether in a coccal or a bacillary form, produced acid—slight in 24 hours, more marked in 48 hours.
3. Milk. There was no clotting, but acid was produced, then alkali, and then acid again, a curious alternating reaction being obtained.
4. Litmus whey. The coccal form from serum cultures produced acid as did the bacillary forms from agar cultures, but to a less extent.
5. Mannite broth. In 1 per cent. mannite broth with Durham's tube, the cocco-bacillus produced neither gas nor acid.

[1] Nuttall, G. H. F., and Graham-Smith, G. S. (1908), *The Bacteriology of Diphtheria*, Cambridge

6. Glycerine-blood-agar. In 1910, Mandelbaum and Heinemann[1] described a special plate method for distinguishing the true *B. diphtheriæ* from pseudo forms. It was thought well to apply this test to our cocco-bacillus, and it was found that it presented colonies in all respects like those described and figured by the German observers for the true Klebs-Lœffler bacillus.

Inoculation of a guinea-pig

A single inoculation experiment was performed, 1 c.c. of a 24-hours' culture grown at 37° C. in *sugar-free* broth being inoculated on December 30, 1909, subcutaneously into a *young* guinea-pig weighing 40 grammes. The broth was prepared by Spronck's first method.[2] There was no effect locally or constitutionally. Although, as a rule, the chief stress has to be laid on the animal inoculations when investigating any diphtheria-like bacillus, still, in this instance, one could not hope to gain much information from them as the strain was derived from a very mild case and was clearly not of a virulent type. Moreover, it had been subjected to the action of formamint and had been sub-cultivated for some time. The man was, indeed, more of a carrier than a patient, although the case, from which presumably he derived his infection through the medium of laboratory cultures, was rather severe. The latter was, however, a complex infection *(vide page* 245). A paper by Arkwright in the *British Medical Journal* for November 12, 1910, on diphtheria carriers and bacilli allied to, but distinct from, the true Klebs-Lœffler bacillus is well worthy of study in relation to this case.

So far as morphology and cultural characteristics go this cocco-bacillus was undoubtedly *B. diphtheriæ*, but I found it hard to believe that two distinct forms—one coccal, the other bacillary—could exist, and that each to some extent could be transformed by appropriate cultivation into the other. The fact remains that such was the case. So far as I can see there was no possibility of error, and Captain Archibald, who on his return from leave, saw something of the work and all the microscopic slides, is satisfied that the facts are as stated. It will be evident that it is important to confirm or refute these observations, for, if cocci alone were found in a throat swab, a case of diphtheria might easily be overlooked. So might carrier cases, for it would appear that the organism tends to assume the coccal form only under certain conditions, one of which may possibly be a diminution in virulence, and another, a free supply of oxygen or the special reaction of the culture medium. It is interesting to note that the whole question of variation in bacteria is coming prominently to the front and one of the most variable is *B. diphtheriæ*. This question is much too large to be discussed here. Reference is made to it in our Second Review, but one may note a paper by Bordet and Sleeswyk[3] on the changes induced by cultivation on different culture media, and the recent researches of Cappellani[4] who has found in the throats of diphtheria patients filamentous forms which on culture change to the classical rod shape. The two German papers, already mentioned, are of special interest and describe a condition resembling in some respects that found in the Sudan. I did not see them until after this paper was in print.

Variation in diphtheria bacilli already recorded

I now give in greater detail a list of the observations made from day to day on the cultural forms of this cocco-bacillus.

December 15, 1909. Swab. Film yielded only Gram-negative cocci with faint metachromatic granules.

[1] Mandelbaum, M., and Heinemann, H. (1910), "Beitrag zu Differenzierung von Diphtherie-und Pseudodiphtherie bacillen." *Cent. für Bakt. Orig. Bd.* 53, *H.* 3.

[2] Quoted in *The Bacteriology of Diphtheria, loc. cit.*

[3] Bordet, J., and Sleeswyk, J. (June 25, 1910), "Sérodiagnostic et variabilité des microbes suivant le milieu de culture." *Ann. de l'Inst. Pasteur.*

[4] Cappellani, S. (1910), "Sulle ramificazioni del bacillo di Löffler." *Ann. Ig. sperim*, Vol. xx., No. 3.

December 16, 1909. Serum cultures from swab. Pure growth of Gram-negative cocci forming small, flat, discrete colonies very like those of *B. diphtheriæ*. Neisser + but faint. Gram both + and — so far as granules were concerned.

December 17, 1909. Agar culture from serum. Within 24 hours Gram + clubbed and beaded rods had appeared, and were found along with the Gram — coccus. Both forms were mixed in masses. Many of the rods resembled typical *B. diphtheriæ*, a considerable number being of the diplo-bacillary type, to the illustration of which, in Nuttall's and Graham-Smith's text-book, reference has already been made. Some of the rods were nearly or completely Gram — but the majority retained the stain.

A confirmatory serum subculture, made at the same time, showed only cocci. Some curious swollen-looking forms were present.

December 18, 1909. 48-hours' agar culture. Same rod forms seen. No cocci. A certain number of the bacilli presented a well-marked beaded appearance. At this stage some rather suggested Hofmann's *B. pseudo-diphtheriæ*, possibly owing to the progressive change in morphology they were undergoing. No true metachromatic granules were obtained on staining with Neisser.

December 19, 1909. 72-hours' agar culture. First appearance of barred and of involution Gram + forms. Three distinct types present. Stout Gram + rods and Gram — rods and Gram—cocci some of which, however, showed distinct dark granules. A 72-hours' broth culture exhibited much the same appearance, but most of the rods were Gram —. Beaded rods were marked (Plate XIV., fig. 2).

December 20, 1909. 96-hours' agar culture. Further increase of bacillary forms. Involution forms more marked. Nearly all the rods Gram +. Beaded and barred forms much in evidence. On staining with Neisser no definite metachromatic granules obtained, but it was found that transference to serum plates resulted in the appearance of Neisser's dots which in the majority of the rods were bipolar.

December 22, 1909. The observations were made every day, but one may pass to the 144-hours' agar culture. It proved practically wholly bacillary, the great majority of the rods being also Gram +. Involution forms of the clubbed type assumed by *B. diphtheriæ* appeared after 248-hours' growth on agar, and were marked in a 288-hours' culture, which showed various swollen and irregular forms as well. Typical barred and beaded forms still persisted as did coccal forms, and it was a point of interest that some of the Gram-negative rods looked as if they were reverting to the diplococcal form. A serum tube culture of the same age presented a very similar appearance.

From this date onwards, involution proceeded apace. Amorphous masses were found in films stained by Gram and prepared from a 432-hours' old agar culture. The changes were like those seen in *B. diphtheriæ* undergoing involution and degeneration, and the last culture examined in this series was a 672-hours' old agar growth of January 13, 1910, which showed, when stained by Gram, extreme degeneration of the bacilli, scarcely any recognisable bacilli being left, but the dark stained polar bodies still showed up well. The few coccal forms remaining were much swollen.

At a later period, time permitting, a few further observations were made.

March 1, 1910. An old agar culture made on February 5, 1910, was found still to be alive. It showed Gram + involution forms, many clubbed forms, and a few Gram — rods. No cocci were found. A subculture was made on agar, and, after 48 hours, one was astonished to find an almost pure coccal growth, the organism being for the most

part Gram —. Controls were immediately made — the February 5, 1910 agar culture being subcultured on to serum and agar.

The former, after 24 hours, showed short, thick Gram + bacilli and some Gram — forms. One Gram — rod with faint terminal polar bodies noted. There was a distinct tendency to coccal formation, such coccal forms being Gram +.

The latter, after 24 hours, showed bacillary forms like those on the serum. It was found that the agar used for the first subculture had been prepared from Doerr's dry medium and was neutral in reaction, while in the second case the ordinary + 10 agar had been employed. Some of this Doerr's agar was then taken and standardised to + 10. A culture of the February 5, 1910 growth on this, showed Gram + and Gram — rods, chiefly the former. Many of the latter exhibited polar bodies and closely resembled typical *B. diphtheriæ*.

Another subculture on neutral agar showed the tendency to coccal formation, so apparently the reaction of the medium has something to do with the form assumed, albeit Cobbett's work on *B. diphtheriæ* did not show it to possess any sensitiveness in this direction. One should note, however, that, as Edwards[1] has recently pointed out, evidence is accumulating to show that a bacillus indistinguishable morphologically from the Klebs-Lœffler organism and to be distinguished from it culturally only with an uncertain degree of accuracy exists more commonly than was supposed. This may possibly be the bacillus which Millard found gave a delayed growth in culture, *i.e.* only appearing after 48 hours' incubation, a most important point as regards diagnosis.

It is perhaps unnecessary to discuss the question at greater length but this paper will have served its purpose if it induces observers in the Tropics, at least in dry climates, to be on the look-out for abnormal forms of the Klebs-Lœffler bacillus both in the human throat and upon the media used for isolation and cultivation. Personally, I believe that a coccal form of the true *B. diphtheriæ* does exist in the Sudan and that under certain conditions it is capable of changing into the typical beaded and barred rods which eventually pass into the clubbed and swollen involution forms so characteristic of the Klebs-Lœffler organism.

Additional Note

The following are the details of the case from which, in all probability, the infection was originally derived. The clinical notes were kindly sent me by Dr. E. S. Crispin, Assistant Director, Sudan Medical Department. It is of interest from two points of view.

1. The presence of Klebs-Lœffler bacilli was masked by the presence of a spore-forming organism of a septic type.

2. This organism was probably responsible for a peculiar ulcerative condition of the mucous membrane of the palate, tongue, gum and cheeks.

A British Officer infected at Khartoum

Case of English officer, aged 46, found to be suffering, on November 27, 1909, from a follicular tonsillitis of the right tonsil, and a rather large patch on the left. Temperature 100° F.

Treated by formamint and a mixture containing potassium chlorate and cinchona bark. *November* 28, 1909. No improvement. Temperature 99° F. Patch on left tonsil larger. Swab taken and sent for examination. A gargle of potassium chlorate and carbolic acid was substituted for the formamint.

[1] Edwards, A. D. (August, 1910), "The Increasing Difficulty of the Diphtheria Carrier." *The Practitioner.*

(Films made from the swab showed no cocci. At first nothing was found except the large spore-bearing organism to be described, but prolonged and careful search revealed the presence of a few but quite typical *B. diphtheriæ*. They were very nearly overlooked. The cultures on Lœffler's blood serum yielded only the former organism which grew rapidly, and completely obscured any colonies of *B. diphtheriæ* which may have been present.)

November 29, 1909. No improvement. Follicular tonsillitis still present on the right side. No change in patch on left tonsil. An ulcer with a bright red margin has appeared on the hard palate and another under the tongue. The bases of these ulcers are covered with a dirty greyish-yellow slough.

The report on the previous days' examination having been received, 2000 units of diphtheria antitoxin were given subcutaneously about 7 p.m. The ulcers were swabbed and so was the throat.

(Film from the ulcer swabs showed the large rods only, those from the throat swabs showed a double infection but, as before, *B. diphtheriæ* was present in very small numbers. In cultures from the swabs *B. diphtheriæ* was lost owing to the profuse and rapid growth of the larger organism. Plate cultures failed to separate out the two bacilli.)

November 30, 1909. Patient feels very much worse. Voice very weak and husky. Considerable pain at seat of injection probably owing to the antitoxin being rather old. Throat slightly better. The question of preparing a vaccine from the spore-bearing organism was discussed, but it was decided that it would not be safe to administer any such vaccine, and it was recognised that the great depression was due, in the main, to the diphtheritic poison. Enquiries elicited the fact that some days previously patient had been in the company of an officer who had recently suffered from diphtheria and may have been a carrier case. In the evening there was a distinct improvement. The patch on the left tonsil was smaller. A fresh ulcer was found to have appeared on the lower gum of the right side.

Another 2000 units of antitoxin given.

December 1, 1909. Patient distinctly better. Pulse very slow but of good tension. Several fresh angry-looking ulcers have appeared on the buccal mucous membrane of the left side. The other ulcers are improving.

December 2, 1909. General improvement maintained. A biniodide of mercury mixture ordered. Swabs taken.

(No Klebs-Lœffler bacilli found. Other organism still present.)

December 3, 1909. Patient much better. One of the ulcers was touched with silver nitrate.

December 4, 1909. Progress very satisfactory. Ulcers on the gum and under the tongue healed, others in a healthy state. Fresh swabs taken. (Only the spore-former present.)

A tonic was now ordered in addition to the mouth-wash.

From this date onwards a gradual improvement took place, the mouth and fauces being clear and free from all bacillary infection by December 9. Patient went to his office on the 11th, but felt so weak that he was unfit for work. Accordingly he left Khartoum for a change and sea-trip which had the desired effect of establishing complete convalescence.

Comments.—The diphtheritic infection need not detain us. There was nothing special about it. Bacteriologically, the infection was slight and yet the rather severe constitutional symptoms, evidenced chiefly by extreme weakness and a tendency to heart failure, must be

laid in the main to its charge. Doubtless, however, the other organism was to blame to some extent and certainly it appeared to be the cause of the unpleasant septic condition evidenced by the presence of the ulcers previously mentioned.

As in such literature as one has been able to consult there is no mention of any organism of this kind causing a similar lesion in the mucous membrane of the mouth and fauces, it seems advisable to place on record such facts regarding its morphology, cultural characteristics and pathogenic action as have been revealed on investigation. Unfortunately, the latter could not be very thorough. There was the usual lack of skilled help, and, like too much of the research work, it was a struggle to get even the few observations made performed in a careful and satisfactory manner. At the time the organism was isolated it was impossible to do more than cultivate it on several media, prepare specimens and inoculate one gerbil. Captain Fry at a later period took up its study after it had undergone sub-cultivation for some time. Unfortunately, he contracted Malta fever and the work had to be abandoned.

Characters of the organism

Morphology. In films prepared from swabs the organism shows as a short stout rod, motile and often a little thicker at the centre than at the ends which are, however, more rounded than pointed. The bacillus stains readily with the ordinary aniline dyes and is found to measure 2·5 μ to 3 μ in length and a little over 1 μ in breadth. Diplo-bacillary forms are seen and a few short threads. No spores were noted in these preparations. The general arrangement of the rods in the film rather suggested *B. diphtheriæ* and this resemblance was heightened by the fact that, after staining with



G. Buchanan × 1000 *diam.*



G. Buchanan × 1000 *diam.*

Figs. 79 and 80.—Organisms from case of British Officer

Gram and counter-staining with saffranin, while the majority of the rods did not retain the stain, a certain proportion did, and these for the most part only in portions of their length so that they presented an appearance of polar bodies or granules (Fig. 80). After cultivation on agar the bacillus is found to be very pleomorphic, occurring as short, stout rods, longer, thinner rods, forms like thick diplo-bacilli and long threads which suggest a leptothrix. It stains readily with the ordinary aniline dyes. When stained with Gram, most of the short forms are found not to retain the stain while the longer forms as a rule do. The threads stain in a peculiar way, some retaining the colour throughout their whole length but the majority holding it only here and there and thus presenting a beaded or broken appearance. Fig. 79 perhaps gives a better idea of the appearances

presented than any description can. There is a much greater tendency to retain the Gram stain than is shown in swab preparations direct from the lesions. The individual
Polar bodies rods and the threads have rounded ends, and the former, in specimens stained with Gram, often exhibit polar bodies at either end. These are not spores for a certain number show oval, unstained spores in addition, centrally placed in the case of the very short stout rods which then rather suggest *B. œdematis*. Some of the rods appear swollen and spindle-shaped, the spindle retaining the Gram stain at either end, while, if counter-stained with saffranin, the body of the spindle has a barred appearance owing to irregular staining. The picture presented is not unlike that of an involuted and barred form of
Morphology *B. diphtheriæ*. Sometimes in such specimens one meets with a faint pink rod thicker at one end than at the other and showing areas of darker staining at either end and at the middle. The size both of rods and chains varies so much that it seems scarcely worth while giving measurements, but the very short rods are about $1{\cdot}5\,\mu$ in length by $\cdot5\,\mu$ in breadth, while longer forms range from $3\,\mu$ to $9\,\mu$ in length and even more. Beyond this magnitude, however, the appearance is rather that of filaments which are unbranched but often sinuous and frequently attain a length of $20\,\mu$ and more.

Owing to irregular staining and pleomorphism, the organism, in a field stained with Gram and counter-stained with saffranin, presents a fragmented appearance, some of the very small rods looking like chips broken off the longer bacilli or the filaments. Sometimes a thread appears studded over here and there with small dark granules, a different appearance from the above-mentioned beading. Generally speaking the organism recalls one of the group of spore-forming, septic, pathogenic anærobes of which *Bacillus œdematis*, *Bacillus welchii* and *Bacillus cadaveris sporogenes* are notable examples. Unfortunately it was not stained to see if flagella were present.

Cultural characteristics.—The organism is, however, not anærobic—at least it is not an obligative anærobe, while the possibility of its being a facultative anærobe has not been tested, but is somewhat discounted by its very free, rapid growth under ærobic conditions. In broth it produces turbidity, forms a scum and is very foul-smelling with an odour suggestive of putrefaction, a characteristic true also of its growth on agar and blood serum.

On agar slope it produces a "crinkly" or wrinkled yellowish growth which speedily turns brown, and soon spreads all over the surface of the slant. Blood serum is slowly but completely liquefied. Gas is produced in lactose broth, but neither gas nor acid are found in glucose or mannite broth. A pellicle forms in glucose broth.

Not fatal for gerbils *Pathogenicity.*—A gerbil inoculated, subcutaneously, with a small quantity of a 24-hours' broth culture, remained unaffected locally and generally. It had been intended to test the effect of this bacillus on the buccal mucous membrane of animals, and more especially of monkeys, but, as stated, this and other work was not carried out, and these are all the facts which have been collected. Hence, one cannot say if it played a part in the pathological picture.

Medical Officers would do well to be on the look-out for this form of ulceration of the oral mucous membrane and the fauces, to note the constitutional symptoms, to observe if there is any association of the condition with diphtheria, and to take swabs of any suspicious case and send them to the laboratories, where it is hoped this interesting bacillus may one day be more fully investigated than was possible at a time of much stress and severe pressure.

Some Aspects of Tropical Sanitation[1]

by

The Director

When your President did me the honour of asking me to deliver an address before this influential Association, he suggested that it should deal with the subject of Tropical Sanitation. While I hastened to assure him that my time and energy were both very much at your service, I took exception to the title, and for two reasons. In the first place, it would have been presumptuous on my part to deal with Tropical Sanitation as a whole. While I have seen something of hygienic questions in the West Indies, in a sub-tropical portion of South Africa, and in certain regions of Equatoria, my experience has, in the main, been confined to those tawny wastes in which, like a Phœnix from the ashes, has arisen that new city of Khartoum, which, from the attention paid to it by tourists and influential persons during the brief winter season, has been somewhat inaptly termed the new hub of the Universe.

In the second place, while only thirty years ago the time at my disposal would have been more than ample to discuss every aspect of the question, nowadays one cannot possibly do more than touch upon the fringes of a subject which has assumed, thanks to the work of Manson, Ross, Bruce, Laveran, Simpson, Finlay, Gorgas, and others too numerous to mention, amazing proportions, an abiding interest, and an importance which it is well-nigh impossible to overrate. I therefore begged Sir James Crichton-Browne that he would permit me to modify the title and term the address "Some Aspects of Tropical Sanitation," and with his usual courtesy he kindly assented.

I might almost have chosen as the title "One Aspect of Tropical Sanitation," for it seems to me that a gathering like that before me would be more interested in the aspect or point of view of the Sanitary Inspector.

Now, gentlemen, I am not a Sanitary Inspector, but I have had to perform, none too efficiently perhaps, the duties of this onerous office, and so I can speak with some experience on, and I may even say somewhat feelingly of, the subject. I have at the outset a proposition to enunciate, a creed to declare, and in doing so, I speak no longer from the standpoint of the inspector but from that of the Medical Officer of Health.

It is this—that just as the non-commissioned officer is the backbone of the British Army, so should the well-trained, certificated, sensible, honest and energetic British sanitary inspector be the backbone of sanitation in our tropical colonies and dependencies. Personally, I believe that certain failures in anti-malarial measures have been due to his absence. I am certain that an intermediary of his type is required between the principal Health Officer and the subordinates, usually natives, who carry out the tasks which in this country would fall to the lot of white men. I have tried untrained men, I have tried what here you would call foreigners, both the Greek and the Italian, and I have found them unsatisfactory in this class of work. Despite the expense involved I advocated the employment of trained British inspectors, and have, on the whole, had no reason to regret the choice. Everything depends, of course, on the type of man. All are not good, all are not suitable for work in the Tropics, all cannot stand the strain, the disappointments, the necessity for constant alertness, the little worries, for in the Tropics the mole-hill,

The type of man required

[1] Being a paper read at the Conference of The Sanitary Inspectors' Association, at London, on August 30, 1910.

with marvellous celerity, becomes the mountain. If you want to know the kind of man WE want read Sir Havelock Charles's brilliant paper to the tropical medicine section at the recent meeting of the British Medical Association. You will say, as I did, "Surely he describes the admirable Crichton of antiquity; can this paragon be found?" and you will reply, as I did, "Yes, for the old country, even in these so-called degenerate days, still breeds this type of man," a fortunate thing for the British Empire and for the advance of that sanitary science which in the Tropics at least signifies so much in the way of health, energy and material prosperity.

In Khartoum we now have four British inspectors, while I understand that similar appointments have been made in India, that home of tropical hygiene, and elsewhere. I sincerely hope the practice will become universal and that those responsible for framing your curriculum and arranging your examinations will afford facilities for the study of tropical hygiene and set questions—and not too easy questions—for the benefit of those who are willing to profess this branch of knowledge. Furthermore, such men must be well paid. Their posts must be made attractive, they should be pensionable, they ought to get good leave periods, and, if thought desirable, study-leave when that is feasible. Nothing is more progressive than sanitary science, and an inspector who has got a grasp of local conditions and feels himself competent to cope with local problems must not rest on his oars. If he is wise he will remember that his dry system of conservancy will pass away, that his rough-and-ready water-supply will disappear, that his rubbish heaps or home-made incinerators will give place to destructors, that it is essential for him not to forget his knowledge of pipes and taps and water meters and steam blasts and the uses of clinker, but to cherish the memory of these things and to keep himself abreast of all modern views and of all recent advancements. Science is at all times a hard mistress, and sanitary science is especially woman-like in being so exceedingly changeable. You laugh, gentlemen, but remember, it is all the better for it. In Scotland, we have an old saying that changes are *lichtsome*. I confess that sanitary changes are sometimes fearsome as well. To return to our Inspector. Let us suppose we have secured him, even made him sign an agreement, and landed him on the spot, which are his friends and which his enemies?

Need for up-to-date knowledge

The Inspector's friends

So far as the Sudan is concerned, his chief ally is the sun. It was Jennings who, in speaking of the sun in Abyssinia, stated that its power was such that it could well-nigh convert the smell of a pole-cat into the odour of a nosegay. There is here no doubt a little poetic licence but much that is very true. For instance, it has been definitely proved that in India the typhoid bacillus survives exposure to the sun for a much shorter period than it can do under the same conditions in England. Doubtless, its death is as much due to that desiccation, which is so fatal to nearly all the pathogenic bacteria, as to the direct lethal action of the sun's rays and of the sunlight. At the same time too much reliance must not be placed on the sun as an ally, for Harrison has reported the recovery of typhoid bacilli from Indian dust after 77½ hours, during 23 hours of which the dust had been exposed to the direct rays of the Indian sun at Kasauli in the Punjab, where I believe the conditions do not greatly differ from those obtaining in Khartoum. It is perhaps more as a deodorising agent that the sun befriends the Sanitary Inspector. Deposits of excreta rapidly dry up and become inoffensive, though, as Smith has shown, in Benares, and as we have also found in Khartoum, such deposits may yet serve as breeding-places for the house-fly, and not only the excretal masses themselves, but the soil under them to a depth of several inches. Though the sun may thus fail to prevent the house-fly breeding out, he does his kindly work later, kindly, that is, towards

our Sanitary Inspector, but not towards *Musca domestica*, which he slays in large numbers in April and May. I have noticed frequently how rapidly the number of house-flies diminishes once the sun attains his summer power. Sunlight, however, is not only operative on soil and soil conditions; it affects in a very marked manner the bacterial content of superficial waters, and, as you may know, Clemesha, in India, has proposed to employ the effects of sunlight on water bacteria as a means of arriving at a suitable bacteriological standard for such waters in tropical countries. It is a fortunate thing that the bacteria with the most evil significance from a hygienic standpoint, appear to be those most susceptible to the action of sunlight.

The sun an ally

We must, however, remember that there is a great difference between the action of the sun under moist conditions and its effects when the climate is dry. It is only in the latter state that the sun is able to exercise its full beneficial effects, and one of the first things a Sanitary Inspector has to recognise in a country like the Sudan is the difference between the dry and the rainy seasons. It is true that the rainy season in Khartoum is, as a rule, trivial, but every now and then the rains become for a time torrential, and, with a soaked, though rapidly drying, soil, a humid atmosphere, and a considerable temperature, bacterial activity is stimulated, and it is well if, at the same time, the activity of the Sanitary Inspector increases to keep pace with, and eventually surpass, that of such pathogenic organisms as the *Bacillus typhosus* and the *Bacillus dysenteriæ*.

Influence of humidity

The sun, then, on the whole is a good friend. Sad to relate, he must also be classed amongst the enemies, for his effects on the Sanitary Inspector himself are not always beneficial. What tropical resident has not, at times, dreaded the molten mass mounting rapidly to his zenith, and has not breathed a sigh of relief when old Sol, for one night only, has taken his last peep over the edge of the world? During the long summer, from April to November, the conditions, save during July and August, are undoubtedly trying. The sun's heat is exhausting, the sunlight is apt to play havoc with the nervous system, there is the risk, a slight one under dry conditions, of sunstroke and of sunheadache. One has to spur oneself to exertion, and the best of men is apt to flag. The sun becomes a foe, but he may aid the Medical Officer of Health at such times by enabling him to weed out the fit from the unfit, by acting as a test of efficiency and devotion. Next to the sun, dryness and lack of rain, as already indicated, are good allies. In the Sudan the character of the population is also a favouring factor. The people, if treated with tact and discretion, and if their few religious and racial prejudices are respected, are easy to manage. There is no enmity towards the Sanitary Inspector. He is known as a benefactor, and it is rare to find squabbles and disputes arising. We cannot practise the militant hygiene of Panama, and there is, as a rule, no need for stringent measures. In part this is due to apathy, in part to the habit of obedience inculcated in those living under British rule, and in part to an intelligent understanding and a desire to co-operate in work which is for the public weal.

Native populations and their attitude to the Inspector

Above all things, we have no cultured fanatics. The anti-vaccinationist has not yet raised his voice in the desert. The native is intelligent, and readily appreciates the value of Jenner's great discovery, for to him for many years small-pox was a dread calamity, while now it is becoming well-nigh as extinct as the dodo, or at least as rare as the whale-headed stork of our southern swamps.

Still, the apathy already mentioned leads me to class the character of the population also under the foes of the Inspector. His chief difficulty is with the Egyptians and with low-class Greeks and Italians, for the Arab and the black Sudani are cleanly races, and a Sudanese location is usually a model of cleanliness if not of comfort. The habits of

bribery and corruption so prevalent throughout the East must also not be forgotten in the list of foes.

Last amongst the Inspector's friends I hope I may place the Medical Officer of Health. He must train and aid the Inspector. He must look well after his interests, he must not press him too hard, and he must seek to reward good and faithful service. He must also be prepared to back him to the uttermost, so long as he is confident that the Inspector is in the right and is acting with due courtesy in any contest which may arise.

The Inspector's enemies

As regards enemies, besides those already noted, we may mention the prevalence of insect life, but this leads us to speak at once of the communicable diseases, which it is the Inspector's main duty to prevent, to keep in check, and to abolish.

Malaria

First and foremost stands malaria. An Inspector has scarcely settled down before he is haled to the Wellcome Tropical Research Laboratories and instructed in the mysteries of mosquito life and propagation. The instructions need not be very extended or very minute. Three species of mosquito alone concern him closely—the malaria-carrying *Pyretophorus costalis*, the ubiquitous and well-haunting *Culex fatigans* and the dweller on board steamers and boats, the breeder in "zeers" and domestic utensils, the black-and-white, vicious tiger mosquito, *Stegomyia calopus* or *fasciata*, which in the New World has gained so unenviable a notoriety as the carrier of the virus of yellow fever. The Inspector must speedily familiarise himself with the water stages of these three insects. He must know, above all things, their respective larvæ, and should be able at once to "spot" their respective eggs and pupæ. He must know their habits and their distribution in the town. He must learn how to combat them, and he must never weary in well-doing.

We learn by our failures, and I can, perhaps, best illustrate this most important work by a few tales regarding it. Those who desire further information regarding the campaign in Khartoum and its results may consult our laboratory Reports, and the chapter in *The Prevention of Malaria*, by Professor Ronald Ross.

As the Blue Nile falls, pools are left in its sandbanks, and in these pools Anopheline larvæ speedily make their appearance, the mosquitoes invading the precincts of the town from outside, or being conveyed into it by steamers or boats. Now, just above the British Barracks, extensive sandbanks formed, pools were left, and in these pools Anopheline larvæ were found. The pools were treated, but whenever fresh ones formed they became infected. It was evident that *Pyretophorus costalis* was in the neighbourhood, though not a single adult mosquito could be found. The British soldiers were in the habit of wandering along the river bank near these pools. They belonged to a regiment which had suffered from malaria elsewhere. The weather was cold. What happened? An old case relapsed, and immediately several cases of primary infection occurred, nine in all, I think, and, fortunately, not of a severe type. I could not find out whence the Anophelines were coming. On the other side of the river from these pools there existed an area of irrigated land, a thorn in the flesh, but it was always closely watched, and I was assured by the man in charge that all was well there. Such inspections as I made confirmed his statement. I was forced to believe the mosquitoes were breeding out in pools higher up the river, which, owing to their distance from the town, could not be controlled. Suddenly, however, the Sanitary Inspector himself, who lived near the farm, fell ill. I found he had malaria of the same type as that in the British soldiers. A very thorough search all over the irrigated area was at once instituted, and Anophelines were found breeding in several channels

A story with a moral

and in pools amongst the standing crops. The Inspector had relied on the native Inspector, and I had relied too implicitly on the Inspector, and meanwhile the north wind was blowing anophelines half a mile and more across the river, with the results stated.

The moment things were put right at the farm the infection of the pools ceased, and no more malaria troubled the troops. The motto of the British Inspector must ever be, "Do not put too much trust in the native Inspector, however efficient he may usually be."

In this instance the Medical Officer of Health caught out the Sanitary Inspector, so it is only fair to present the reverse side of the case.

One morning a female Stegomyia had the impertinence to bite me on the hand in my office at the Gordon College. Next day the insult was repeated. I was much surprised, for we are far from the dockyard, which is, as a rule, the only place where these mosquitoes are now to be found, and there only very occasionally.

Sending at once for an Inspector, I asked him to make a careful search in the grounds of the building. He did so, and he reported that he had found nothing. He then expressed a wish to inspect the laboratories. I said, "By all means; but you'll find nothing there." I followed him into our bacteriological room, and, with unerring instinct, he made for a "burma," or red earthenware vessel upon a stand. "Oh," I remarked, "you need not trouble to look there; it's always empty."

The Inspector's revenge

"Not of mosquitoes, doctor," was his reply; and then, gentlemen, the murder was out. There were soda-water bottles lying in water to keep them cool in that "burma," and the water had not been changed for days, and was swarming with a lively brood of Stegomyia "wrigglers" and nymphs, while new-born "imagines" were clinging to its sides prior to their first flight upon the blood quest. The culprit was a clerk, who, against orders, had adopted this method of storing his drinks, and had taken no precaution against mosquito invasion.

I fined him, but I had also to fine myself, for I was primarily responsible, and I do not doubt but that in the Inspector's mess there was much joy over my delinquency. The Inspector has to be very wide awake, for mosquitoes sometimes breed in queer places. I remember a high official complaining to me that he was having a bad time with Stegomyia. Mosquitoes of this genus had recently been brought into Khartoum in large numbers by the river steamers, and had, I am inclined to think, been the cause of a small epidemic of horse sickness. This, however, by the way. As no breeding places could be discovered, I was nearly driven to the conclusion that these mosquitoes were survivors of the invasion, for as you probably know, *Stegomyia calopus* is a long-lived insect. However, I fortunately asked the Inspector, a temporary, inexperienced man, if he had examined the rooms as well as the verandah. He had not done so, and further search revealed the presence of larvæ in the water contained in small tin receptacles in which the legs of the ice-chest stood as a protection against the thieving propensities of ants. Recently I found a similar state of matters in the Mess at Wau, in the Bahr-El-Ghazal Province.

An unexpected breeding-place for mosquitoes

One more illustration and I have done. A certain Company took up the question of converting the "Sudd," that matted growth of papyrus, vossia grass, reeds and rushes, into fuel, and sent a steamer and old ferry-boat south to experiment. One morning our Inspector at Khartoum North rang me up in alarm and well-nigh in despair. The ferry-boat had returned full of "Sudd," and full also of mosquitoes, which were flying ashore in a black cloud, seeking whom they might devour. Prompt action was necessary.

The Inspector was ordered to engage special men on the spot, take the laboratory steamer, and tow the offending craft down the Nile to the lonely and sandy Tuti Island, where the cargo was to be discharged and the holds oiled. It was a cheerful task. The ferry-boat had no helm, and she and the steamer went waltzing down stream, while the unfortunate experimenter tore his hair in despair, and eventually invaded my sanctum to find out how he really stood, and to learn the enormity of his methods. I must say he was most reasonable. We were able to meet him half-way, and the threatened invasion was averted, but these episodes will show you how varied are the duties of our inspectors when on mosquito prevention work. As regards other diseases, I need say little. I have mentioned small-pox. One has always to be on the watch that, during chicken-pox epidemics, small-pox cases may not occur and be missed. Chicken-pox, as in India, tends to occur in April and to be a disease of adults—a point of considerable importance.

Small-pox cases may be overlooked in chicken-pox epidemics

As you know, in unvaccinated communities small-pox reverts to its old type and becomes a disease of infants and of children, one sure proof of the preventive action of vaccination. Happily the native is now well protected and small-pox is rare, but it may be introduced, and then measures must be taken similar to those employed elsewhere. I recall a romantic incident in connection with a case which will give you an insight into another aspect of the inspector's life. If you will you may call it the temptation aspect.

A case of small-pox had occurred in the town, and on inquiry it was found that the infected house had been visited by someone from another dwelling. It was necessary to inspect the inhabitants of the latter. I did so in person, accompanied by the "Mamur," as the Sub-Governor is called. The door was opened to us by an old, wrinkled, and blear-eyed crone, who, on hearing our mission, forbade us entrance. "The master of the house was absent," she said, "and we must not intrude." It was explained to her that all that was required was to feel the pulses and look at the tongues of the inmates, but the duenna was obdurate in the extreme! Arguments, promises, and entreaties all proving useless, the Mamur took matters into his own hands and entered the outer hoosh or yard. A wooden door guarded the inner yard—a holy of holies. Despite the ancient hag, this was at last pushed slightly ajar, and first of all an aged, white-haired man presented himself, held out a skinny wrist and protruded a tremulous but otherwise normal tongue. Then, after further delay, there appeared a dainty little figure clad in black, with eloquent, and I am inclined to think, interested black eyes, looking out over the "yasmak," or married woman's veil. The situation was carefully explained to this pearl of great price, and a dainty wrist was forthcoming and then the folds of the silken veil were drawn slightly, very slightly, aside, and a tiny pink tongue appeared, undeniably shapely, undeniably healthy. Satisfied, I beat a retreat, still regarded with suspicion by the she-dragon. Now, while a staid and married Medical Officer of Health may face such a situation with impunity, I ask you how it might have affected a young and susceptible Sanitary Inspector? Truly he has sometimes need of the discretion of a grey beard.

The other diseases need scarcely detain us. Cerebro-spinal fever when it occurs is dealt with as at home. We find formamint lozenges useful. It is possible that Phlebotomus fever, due to the so-called sand-fly, exists in Khartoum as in Malta, Austria-Hungary, and elsewhere. The vector is really a moth-fly or so-called owl-midge, a dipteron belonging to the "Psychodidæ," hairy as Esau, and exceedingly vicious. We have two known species in Khartoum, and, so far, their breeding places have defied discovery, though our entomologist, Mr. King, has suggested they may lay their eggs in the crevices in the sides of wells. If this be so we may yet behold our inspectors hanging, like Mahomet's coffin, between earth and heaven, and diligently engaged in destroying the

Other diseases

noxious broods. It would indeed be a great matter if we could prevent the multiplication of these annoying pests, quite apart from the question of any virus they may convey.

Measles is rare but always troublesome. In my experience it is not so dangerous as in this country, chiefly because the climatic conditions do not favour pulmonary complications. It keeps the Inspector, however, on the alert, as, with early notification, it is sometimes possible to check its spread. Diphtheria is likewise uncommon but is of great interest to the bacteriologist, because I am persuaded a coccal form of the *Bacillus diphtheriæ* exists in the Northern Sudan. I cannot enter into this question here (*vide page* 239). The disease tries the Inspector's patience sorely, owing to the habit common to Syrians, Egyptians, Levantines, Arabs, and Sudanese of relatives flocking to see a sick child. The number of "contacts" is sometimes very great, and it is most difficult to ferret them out. No one responsible for the Public Health feels happy while diphtheria is in our midst. Its insidiousness is only equalled by its virulence, though fortunately antitoxin has abolished half its terrors.

Conservancy methods

Enteric fever and dysentery lead me to speak at once of conservancy methods, and here it is that the Inspector finds the most scope for his energies. In the Tropics, as elsewhere, a water-carriage system of sewage disposal is undoubtedly the best, but such a system is expensive and requires an abundant water-supply, while very often there are special engineering difficulties to be overcome. In the old days, Khartoum was served by the Crowley cart, or so-called "iron-clad." An "insanitary juggernaut" it has been named, and well-named, what with its foul sides, its splashing contents, its ever-leaking lid, its cohort of filthy flies, and its uncertain balance.

A bucket system

It was decided to abolish it, and introduce a bucket system on an approved principle—the buckets having air-tight lids. An apparently satisfactory bucket was found, tested and adopted. When used it is removed and a clean pail placed in its stead. The removal is a daily, or rather a nightly one, and double removal in the twenty-four hours is required in the case of latrines, hospitals, and some other institutions. The covered pails are removed in carts drawn by camels to certain collecting stations, where mule-drawn trollies await them on the conservancy tramways and they are conveyed to the trenching ground. There they are emptied, well cleaned (*vide* Fig. 83), and returned for use.

I am not likely to forget the time when the change was made. Our cleaners are of the lowest class, often drunken, and always inclined to laziness. A change is usually abhorrent to them, new ideas do not penetrate their skulls, they are in many ways like the beasts that perish. We have no special sweeper class as in India, we have to rely on the riff-raff, and they lead us now and then a pretty dance.

I had only one British Inspector at the time, and I advised him to "gang warily," to introduce the new system gradually, but he was ambitious and very keen on the work.

He applied it to the whole town straight away. It was surprising how the cleaners took to it. They preferred its comparative cleanliness, but for a time chaos reigned. I was forced to become an inspector also, to rise at 3 a.m.—and in the winter it is often bitterly cold at that hour—to pursue defaulters whom I found emptying one bucket into another and thereby nullifying all the good of the system, to rush from quarter to quarter, to storm and rave, and sometimes to stand aghast at the pile of pails which had accumulated owing to some accident on the tram line. A crisis occurred. Owing to certain women street-sweepers getting a rise of pay while he did not, every conservancy cleaner struck work. Conservancy was at a standstill. The strike occurred at night. I had a vision of the morning, the empty latrines, the frantic populace. There was only one thing to do. I authorised the Inspector to promise every man a rise of pay, and then

A crisis

I went, hunted up the Financial Secretary, painted the situation in lurid colours, and obtained financial approval. The evil was averted, but the Inspector had to labour like Hercules to get things set agoing in time.

Such a system prevents the soil in the neighbourhood of houses becoming fouled, and, properly conducted, is very efficient. It has, in my opinion, led to a notable reduction of dysentery in Khartoum, while in a population of about sixty thousand we have only some fifteen cases of locally-acquired enteric fever a year. It is somewhat expensive to work, and its success depends almost wholly on efficient inspection. We have a saying in Khartoum that when things are going very smoothly it is well to be suspicious. Rapid work usually means faulty work, for the cleaner is up to every kind of trick, and loves to lighten his load. He has to be taken unawares and promptly punished, but he must be justly punished, for the least injustice rankles, and may speedily bring about a rebellion. I cannot describe all the aspects of this work, but let us look for a moment at the method of disposal of the excreta.

Those of you who have read that masterly study of social life, *Les Miserables*, may remember the fine passage in which Victor Hugo deals with the sewage question:—

A quotation from Victor Hugo

"Paris," he says, "casts twenty-five millions of francs annually into the sea, and we assert this without any metaphor. How so and in what way? By day and night. For what object? For no object. With what thought? Without thinking. With what object? None. By means of what organs? Its intestines. What are its intestines? Its sewers; 25 millions is the most moderate of the approximate amounts given by the estimates of modern science. Science, after groping for a long time, knows now that the most fertilising and effective of manures is human manure. The Chinese, let us say it to our shame, knew this before we did; not a Chinese peasant, it is Eckeberg who states the fact, who goes to the city but brings at either end of his bamboo a bucketful of what we call filth. Thanks to this human manure, the soil in China is still as youthful as in the days of Abraham, and Chinese wheat yields just one hundred and twenty-fold the sowing. There is no guano comparable in fertility to the detritus of a capital and a large city is the strongest of stercoraries. To employ the town in manuring the plain would be certain success, for if gold be dung, on the other hand our dung is gold. What is done with this golden dung? It is swept into the gulf. We send at great expense fleets of ships to collect at the Southern Pole the guano of petrels and penguins, and cast into the sea the incalculable element of wealth which we have under our hand. All the human and animal manure which the world loses, if returned to land instead of being thrown into the sea would suffice to nourish the world. Do you know what these piles of ordure are, collected at the corner of the streets, those carts of mud carried off at night from the streets, the frightful barrels of the night man, and the fœtid streams of subterranean mud which the pavements conceal from you? All this is a flowering field, it is green grass, it is mint and thyme and sage, it is game, it is cattle, it is the satisfied lowing of heavy kine at night, it is perfumed hay, it is gilded wheat, it is bread on your table, it is warm blood in your veins, it is health, it is joy, it is life! So desires that mysterious creation which is transformation in earth and transfiguration in heaven —restore this to the great crucible and your abundance will issue from it, for the nutrition of the plains produces the nourishment of man. You are at liberty to lose the wealth and consider me ridiculous into the bargain—it would be the masterpiece of your ignorance. Statistics have calculated that France alone pours every year into the Atlantic a sum of half a million. Note this, with these 500 millions, a quarter of the expenses of the Budget would be paid."

Great words, gentlemen! No doubt the poetic licence is again visible, but who will deny the main truth of the argument, albeit Victor Hugo ignores the effect of the sewage on fish life, a subject of special interest to us to-day. Furthermore, he does not mention the problems which await, the difficulties which beset, the scientist who would carry this dream into practice. If these difficulties are great in Europe, they are much greater in the Tropics, where money is not plentiful and trained engineers are few. In one respect, however, the Tropics often score. Land is usually plentiful and cheap. Hitherto, at Khartoum, we have had to be content with pitting our sewage in shallow trenches and leaving it to the action of those saprophytic organisms which speedily transform it from an abomination into a harmless product of bacterial activity. We have little rain, and hence cannot grow rain crops on the ground, and in the Sudan, water spells money with a big "M." All the time, however, we have been experimenting on a small scale to see if a septic tank system can be applied to such a dry system of conservancy as is in vogue. It is far from being really dry. The population is in the main Mohammedan, and the Mohammedan, in this respect advanced beyond his western brother, employs lavage, so that the contents of our pails are for the most part semi-liquid. At the same time it was found necessary to dilute the sewage to obtain anything like a good effect. Such dilute sewage was treated both in metal and in brick tanks, ærobically and anærobically, and certainly it is remarkable how quickly liquefaction occurs when the temperature is high. I cannot enter into details, but I recall an incident whereby an Inspector proved his worth. A tankful of diluted sewage had been left to work out its own salvation for 48 hours. It was far from savoury, but I wished to see and obtain samples of the effluent. The outlet valve was rusty, and in order to loosen it we had to employ a key and hammer. These instruments performed their duty with a vengeance, for suddenly the whole valve came away, and there was a rush of foul-smelling effluent of the colour, if not the consistence, of pea-soup. The Sudanese foreman fled. I confess I skipped out of the way with alacrity, only the Inspector stood firm. Knee-deep in the stream he searched for, found the valve, rammed it home, and stayed the flood.

Experiments in bacterial treatment of sewage

"Familiarity," you may say, "breedeth contempt." No doubt; but I said to myself, "This is the man for my money," and I have had no reason to alter my opinion.

We are now about to advance and make a serious effort to start a sewage farm, whereon we may grow fodder crops, such as gerawi grass and burseem. In a country where intestinal parasites are common, vegetables for human consumption should not be produced on sewage-treated soil.

Proposed sewage farm

Personally, I think the scheme will prove successful, and at any rate it is in the right direction, while the fact that our trenching ground is limited, and is in danger of becoming sour, renders some such change imperative.

One has outlined the conservancy method for the main town, but such a system cannot be applied to the long line of native villages south of the town where, tribe by tribe, live hundreds of native labourers and their families. Nor can it be applied to certain extensive native locations in Khartoum North. It is in such cases that the small incinerator, Fig. 81, proves itself of value. It is built of sun-dried brick, protected by a coating of what is termed "zibla"—a mixture of mud, straw and manure, which hardens and withstands rain—is cheap, easily constructed, and easily worked. The man who attends to the fire acts as sweeper over a certain area. The natives follow their usual custom of easing themselves in the open, and the scattered excreta are swept up daily, along with the general refuse of the villages, and burned to ashes, the latter being buried or employed in filling up inequalities in the ground. This method, originally, I think, devised

Incinerators

in India, is of great service, and is a decided improvement on the old Mosaic custom where (as we read in Deuteronomy) :—

"Thou shalt have a place also without the camp, whither thou shalt go forth abroad ; and thou shalt have a paddle upon thy weapon, and it shall be when thou wilt ease thyself abroad, thou shalt dig therewith, and shall turn back and cover that which cometh from thee," a practice very excellent in itself, but impossible of realisation on a large scale in the case of settled communities such as those with which we have to deal.

You will see that in many ways our problems differ from those you have to face in England. To judge from current literature, one of the most serious of the latter is the progressive lowering of the birth-rate, while the whole question of child-life and early physical training is attracting earnest attention. As you are aware, your President is one of the leading authorities on these matters, and on the equally important question of the people's dietary.

The birth-rate

In the ancient town of Haddington many years ago a worthy gentleman lost a maiden aunt to whom he had been sincerely attached. I do not know whether the lady left him a legacy, but certain it is that he erected a tombstone to her pious memory, and had graven on it a verse. You will agree with me that his qualities were more of the heart than of the head, for this is what he wrote :

"Here lies Miss Gourlay, I shall not say what,
But all that a woman should be she was that."

It happened that the fine new tombstone caught the eye of the old parish minister as he was taking a daunder in the kirkyard. He read the epitaph, and shook his head : "Na, na, Miss Gourlay," he said, "this'll no dae." Taking a piece of chalk from his pocket he wrote underneath :

"A woman should be baith a wife and a mither,
Miss Gourlay was neither the t'ane nor the tither."

Now, I tell this little story to assure you that this wise old man would have been amply satisfied with the female population in the Sudan.

It is, I believe, the case, and a matter of interest to anthropologists, that crude contraceptive measures are practised at times by the Sudanese, but such can play no great part in their social economy. One has only to look at the swarm of merry, black, pot-bellied children on the foreshore at Omdurman to realise that the Sudanese are obeying the old Scriptural injunction, "Be ye fruitful and multiply, and replenish the earth." I cannot give you figures, but there can be no doubt the birth-rate is high, and the sight of all these cheery, fearless urchins in itself justifies, if any justification were needed, the Anglo-Egyptian occupation of the Sudan. Nowadays, these children can grow up with some hope for the future, and without the menace of the slave-raider, the sword, the rope, the famine and the pestilence, which in the days of the Khalifa wrought such dire havoc in the country.

It is this question of the children which brings me to another important factor in the Inspector's life—namely, the control of the milk supply. Most of the native infants are breast-fed, and so far as Khartoum is concerned, the question chiefly affects the older children of the poor Greek and Italian communities. For those who can afford it, excellent milk is available from the Government dairy farm, but its price is high, and most of the milk is derived from native cows and goats. Which is the better, the milk of the cow or goat ? The latter animal possesses two great advantages : it very rarely suffers from tuberculosis, and it is cleanly in its person, thanks chiefly to the special form and consistence of its excreta. It is also said to be a clean feeder. I believe that is

The milk supply



G. Buchanan

Fig. 81.—Small Incinerator at Daim (Village)



G. Buchanan

Fig. 82.—Refuse Trolley and Incinerator at Sewage Farm



G. Buchanan

Fig. 83.—Bucket Cleaning at Sewage Farm



G. Buchanan

Fig. 84.—Bucket Repairing

true of the goat in England, but it is certainly not the case as regards our little Sudanese goats. They love rubbish heaps, and I have seen them devouring dirty paper and fragments of women's cast-off clothing. Truth to tell, there is precious little for them to eat in the neighbourhood of Khartoum. Their means of sustenance is to me a mystery, and I am half inclined to think they enjoy chewing tin cans and the fragments of broken bottles. In the Sudan, where animal tuberculosis, unlike human tuberculosis, is very rare, and where cattle live in the open, I think cow's milk is preferable, but this is a matter of taste. The milk from the native herds is very good. Analyses by our chemists show it to be well up to European standards, and to be specially rich in fat. It is not the milk which is at fault, but the milk vendor. Women bring this milk into the town very early in the morning from the outlying villages, and you will scarcely believe me when I say that some of these villages are nine miles away, so that the vendors have to tramp eighteen miles in all, half the distance with their burden of milk, and all for a very meagre profit. Needless to say, they endeavour to improve matters for themselves, though not for their customers. Their methods are not scientific. Any water will do, and any quantity of it so long as the milk retains a whitish colour. Moreover they were in the habit of conveying the milk in earthenware vessels. Anything more fitted for bacterial growth and activity than one of these "burmas" it would be hard to conceive. They are porous, and usually filthy. A dirty bit of rag stuck in the mouth prevented milk being lost by splashing, and added to it all manner of abomination.

"Watering" the milk

At an early period steps were taken to regulate this traffic. The women were registered and given badges. "Burmas" were forbidden and proper cans substituted. Matters were carefully explained to the ladies, and then the fun began. The cheerful and exhilarating sight was frequently witnessed of the Medical Officer of Health and Sanitary Inspector pursuing, on horseback, one of these dames who had reverted to her old methods—had a "burma" on her head, or had been filling her can from a convenient well. Punishment did good, and things improved—only to relapse. It was impossible to keep a very close eye on them all, and now another scheme is to be tried. It could not be carried into effect until the number of Inspectors was increased. As this has been done, the following measures will come into force shortly:—

Registration of milk vendors

The women, all registered, will bring in the milk in properly covered cans to a central station. There the milk will be measured, and samples taken for analysis from time to time. It will then be transferred to tins owned by the municipality, which will have locked covers, and from which milk can be obtained only through the exit taps. Customers will be notified of the change, and it will be their own fault if they do not see their milk supplied through these taps. Meanwhile, the women's tins will be scalded, cleaned, and made ready for the evening, when they will be given back to the vendors on their return from their milk walks, while the empty municipal tins will be collected, cleaned, and stored ready for the next morning. The scheme sounds simple and effective. It remains to be seen how it will work. I think if the women charge a little more for such good milk there will be no hardship on either side—the public will no longer be cheated, and the risk of diarrhœa and marasmus will be lessened. I have now dealt with the main features of the Inspector's life. He has many other important duties. He has to supervise a railway gang who keep the tramway in order; he is responsible for refuse destruction (Fig. 82); he controls the mineral water factories; he keeps an eye on the markets (Fig. 85), especially our primitive fish market, and the slaughter-houses. House-to-house inspection takes up much time, and is combined with the never-ending mosquito work. We have few noxious trades, but such as exist must be watched and regulated.

Miscellaneous duties



G. Buchanan

Fig. 85.—Exterior View of Portion of Meat Market, Khartoum



G. Buchanan

Fig. 86.—General View of Dockyard, Khartoum North

Our Chief Inspector has established blacksmith's shops, where we repair our sanitary pails, quite a big business (Fig. 84). An eye has to be kept on the conservancy animals, and attempts at the pilfering of fodder checked. The clerical work is heavy, and statistics have to be prepared for the annual sanitary budget. Prosecutions have to be attended and evidence given.

Until recently the Sanitary Inspectors had to undertake the poisoning of stray dogs, for there is always a risk of hydrophobia. They have all manner of "orra jobs," and I assure you they earn their pay. Khartoum and Khartoum North cover a large area, and you will not be surprised to hear that our Inspectors are a mounted force. One insisted on their being supplied with horses or ponies. We only take men who can ride.

I remember saying to one of them on his arrival, "I understand you can ride?" "Well, sir," he replied, "I won't say I'm a horseman, but I have been practising very diligently on an old cab-horse at home." I fear he found the fiery Syrian with which he was presented very different from his Scottish charger; but he soon became an expert, and could now make a jockey sing small.

Like a woman's, an Inspector's work is never done, but he has the satisfaction of seeing the good results of his labours. I have no wish to boast, and know that statistics have to be collected over a long period, but, so far as communicable diseases go, I doubt if a healthier city than Khartoum exists in Africa at the present moment (*vide page* 281). Something is due to its comparative isolation (another of the Inspector's friends which I omitted from the list), but more is due to the fact that the town was caught young, and early in its new career passed under what some might call a sanitary tyranny. Very different are the conditions from those which rendered the old Khartoum a pest spot, and led the wily Dervish to raze it to the ground and establish his capital at Omdurman. A very great deal is due to our Sanitary Inspectors. I sometimes think that when a Medical Officer of Health is complimented on the state of his district he would do well to recall what Lord Howe said to old George III. after the crowning victory of the glorious First of June. On his own quarter deck he received a jewelled sword and the congratulations of his Sovereign. The tears stood in the old admiral's eyes as he turned and stretched an arm forward to where his sea-dogs were clustered: "It is not I, your Majesty," said he, "it is these gallant fellows."

The new Khartoum

Tropical sanitation means war, a ceaseless war, an exhausting war, but, thank God! if properly waged, a successful war against those conditions which tend to slay the white man and the black. It is Ray Lankester who has said that if we could only apply our present knowledge properly all communicable disease could be stamped out in the short course of fifty years. With certain reservations as regards some tropical disorders, I heartily concur in this optimistic view. What we require is education, such legislation as will crush the cultured but ignorant fanatic and aid the worker, a devotion to the cause, and a well-trained band of helpers.

Foremost amongst these stands the Sanitary Inspector, and thus it is that I trust we may soon see him in ever-increasing numbers taking up that white man's burden, which of all burdens is perhaps the noblest to bear.

Then, and then only, may we, in part at least, experience on earth a foretaste of that which we are promised hereafter: "There shall be no more death, neither sorrow nor crying, neither shall there be any more pain, for the former things are passed away."

Sanitary Notes

BY

The Director

We will now consider a little more in detail some of the subjects and problems to which attention has been directed in a general way in the preceding paper.

Following the course pursued in the last Report, attention may first be directed to the meteorological conditions which have prevailed in Khartoum since these were last recorded. The table has been prepared by Mr. J. A. Goodson, our Assistant Chemist, who has charge of all the meteorological reporting work in Khartoum.

Year	Month	Monthly mean maximum °C	Monthly mean minimum °C	Monthly mean humidity per cent. °C	Total rainfall MM.	Highest temperature during the year	Lowest temperature during the year	Prevailing winds
1908	Jan. to July	36·6	20·0	—	1·2	46·1 in June	7·1 in Jan.	N.N.W.
1908	July to Dec.	36·4	22·4	—	150·4	—	—	S.S.W.
1909	Jan. to July	37·4	20·9	—	0·8	44·5 in April	9·8 in Dec.	N.N.E.
1909	July to Dec.	36·8	22·3	—	145·7	—	—	S.W.
1910	Jan. to July	36·8	20·0	—	34·9	46·6 in June	9·5 in Jan.	N.N.E.
1910	July to Dec.	36·3	22·0	—	74·0	—	—	S.S.W.

Reference to the meteorological table given in our Third Report (*page* 63) will show that Mr. Goodson's figures differ markedly from some there recorded and it is possible that in the former table some errors, due to transcribing, occurred. Future observations should clear up this point. The high total rainfall figure for January to July, 1910, *i.e.* 34·9 mm., was almost wholly due to a single, prolonged and torrential shower in June.

Mosquito Work

It is some considerable time since one submitted statistics dealing with the mosquito-prevention work, although these have been carefully kept throughout. Now, however, I submit two tables, one for Khartoum and the other for Khartoum North, which give figures for one complete sanitary year. These are most interesting and instructive, and, though not absolutely indicative of the state of the town, are sufficiently reliable to give an idea of what has been happening. Mosquito "tables"

The small receptacles mentioned comprise buckets, barrels, ice-chests, grindstone-stands, small tanks, water-dishes in fowl runs, water jugs on dressing tables, flower vases, bottles and small dishes of water placed beneath the legs of ice-chests, tables, or food receptacles to check the depredations of ants. In the Khartoum North Table the malign influence of the irrigated land there is noticeable. It will be seen how the

TABLE A

MOSQUITO STATISTICS FOR YEAR ENDING SEPTEMBER 30, 1910

KHARTOUM

Month	No. of Wells, Pits, etc., Infected			No. of Zeers and other small Receptacles Infected			No. of Boats and Steamers Infected			No. of Pools Infected			No. of Permanent Collections in City	Percentage of Infected Permanent Collections	Last Year
	C	S	A	C	S	A	C	S	A	C	S	A			
1909															
October	34	—	5	—	10	—	2	3	—	—	—	3	1195	3·2	5·8
November	33	—	—	—	16	—	—	1	—	1	—	5	1201	2·7	5·3
December	34	—	—	—	16	—	1	—	—	—	—	34	1202	2·8	5·0
1910															
January	33	—	—	—	4	1	—	—	—	—	—	4	1203	2·7	4·5
February	32	—	—	—	5	—	2	—	—	—	—	—	1206	2·6	3·0
March	23	—	1	—	1	—	2	—	—	—	—	—	1206	1·9	3·4
April	27	1	—	—	3	—	3	3	—	3	—	—	1207	2·3	3·0
May	33	—	—	1	—	—	—	—	—	—	—	1	1207	2·7	3·8
June	24	—	—	—	—	—	1	—	—	1	—	1	1207	1·9	2·5
July	22	—	—	—	2	—	—	1	—	—	—	—	1204	1·8	2·7
August	21	—	—	—	3	—	—	—	—	—	—	1	1204	1·7	3·8
September	27	—	2	—	6	—	2	1	1	—	—	1	1203	2·4	3·8
	343	1	8	1	66	1	13	9	1	5	—	50	—	2·4	

C = Culex S = Stegomyia A = Anophelines

TABLE B

MOSQUITO STATISTICS FOR YEAR ENDING SEPTEMBER 30, 1910

KHARTOUM NORTH

Month	No. of Wells, Pits, etc., Infected			No. of Zeers and other small Receptacles Infected			No. of Boats and Steamers Infected			No. of Pools Infected			No. of Permanent Collections	Percentage of Infected Permanent Collections
	C	S	A	C	S	A	C	S	A	C	S	A		
1909														
October	8	—	—	—	—	—	—	—	—	2	—	—	215	3·72
November	8	—	—	—	1	—	—	—	—	—	—	1	,,	3·72
December	7	—	—	—	—	—	—	—	—	1	—	1	,,	3·25
1910														
January	12	—	—	—	—	—	—	—	—	11	—	6	,,	5·58
February	4	—	—	—	—	—	2	2	—	18	—	7	,,	1·86
March	3	—	—	—	—	—	—	1	—	11	—	2	,,	1·39
April	8	—	—	—	—	—	—	—	—	10	—	1	,,	3·72
May...	21	—	—	—	—	—	2	4	—	4	—	2	,,	9·76
June	7	—	—	—	—	—	2	2	—	2	—	—	,,	3·25
July...	7	—	1	—	—	—	2	1	—	7	—	—	,,	3·72
August	16	—	—	—	2	—	2	—	—	17	—	3	,,	7·44
September	6	—	1	—	—	—	1	1	—	13	—	39	,,	3·25
	107	—	2	—	3	—	11	11	—	96	—	62	—	4·22

Includes 94 Culex and 60 Anopheline collections found on the Sudan Dev. Co.'s farm.

town is threatened, and there can be no doubt that if work stopped for two months, cases of locally-acquired malaria would soon be notified, and everyone would be complaining of the voraciousness of Stegomyia throughout the day and the blood-thirstiness and insatiable appetite of *Culex fatigans* in the watches of the night.

Malarial outbreak in Khartoum North

One has mentioned the farm at Khartoum North, both here and in previous Reports, and it may be well to consider a small outbreak of malaria due entirely to faulty conditions prevailing there, and which, as already stated *(page* 252*)*, escaped detection for a time.

If one turns to *page* 277 and looks at the Table, it will be evident that practically all the cases of locally-acquired malaria occurred at a certain period of the year. The reason is not far to seek and is due to the above-mentioned infestation. Moreover, the great majority of the cases occurred either amongst British troops housed in barracks on the other side of the river from the farm, or in persons living not far from the boundary of the irrigated area. It is, however, rather the persistence of the mosquito infection to which I would direct attention. Fig. 88 shows how the land at the farm is laid out, at what parts of it infection occurred and at what dates C. (Culex) and A. (Anophelines) were found. It will be seen that the infection ranged from January 30, 1910, the day on which special attention was directed to the farm as a result of the illness of the inspector *(page* 252*)*, to June 5, 1910, and this despite every effort to stamp it out. The figures, however, it must be remembered, do not refer to places where mosquitoes bred out, but merely to pools, etc., in which the larvæ or pupæ were found, a very different matter. Indeed, it was very rarely that the water stages of the insect got the length of becoming pupæ for the inspections were numerous and rigorous. There was no way of getting at the *imagines*, of which presumably there was not a great number. Indeed, not a single adult *P. costalis* was ever found at the British Barracks, despite the nine cases of malaria occurring amongst the troops. Undoubtedly, some of these infections took place at Buri, to the east of the barracks, at the places where anophelines bred out in the river pools, a locality eventually put out of bounds for the soldiers.

Malaria cases ceased to occur although the infection of the farm continued. It must be remembered that, although, as Ross has pointed out, there must be, in all probability, a considerable number of Culices about before they constitute themselves a nuisance, a single infected anopheline may be quite sufficient to give rise to several cases of malaria. At the same time, as there must be a source of infection, and as the mosquito must find that source in order to become infected, it is probable that, in most cases, if malaria is to be, in any sense of the word, epidemic, anophelines must be present in fair numbers. Hence a few female *Pyretophorus* flitting hither and thither and seeking, desperately, water-collections wherein to lay their eggs need not be regarded with alarm provided such water-collections are under scrutiny. It is, however, a totally different affair if there is no routine and efficient inspection, and a little consideration of these facts and of the plan, should impress upon all concerned not only that constant and unremitting vigilance is essential, but the absolute necessity for the establishment of dry zones.

What may be called the irrigation rules have already been stated,[1] but when, at a later period, the above-mentioned farm changed hands, re-infection again occurred, and there was a recurrence of malaria amongst British troops, fortunately on a very small scale, it was found advisable to supplement the rules by a few additional regulations. As these are of practical interest I give them here, though in certain particulars they overlap the more general instructions:—

Additions to the "irrigation rules"

[1] *Third Report, Wellcome Tropical Research Laboratories*, 1908, p. 67

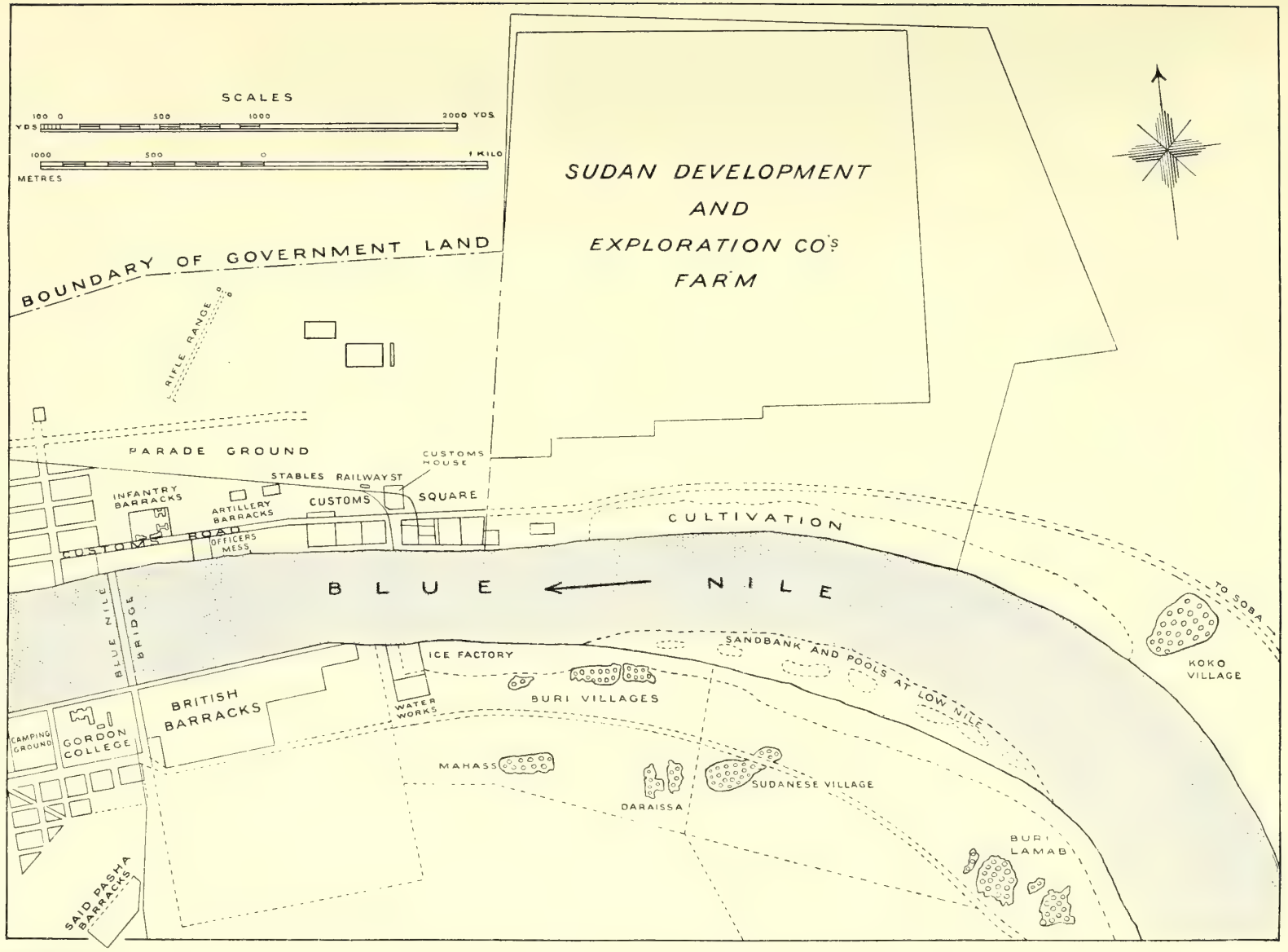


FIG. 87.—PLAN OF KHARTOUM, SHOWING POSITION OF SUDAN DEVELOPMENT AND EXPLORATION COMPANY'S FARM

Main gudwals

1. The main gudwal must be examined for leakage at least three times a week. As soon as leakage is discovered, the fault must be repaired at once and the water dried off the ground.

2. All inlets to branch gudwals must be properly closed when the channels are not in use.

3. The water at the ends of main gudwals must be cleaned every day, as all the refuse, such as melon skins, grass, etc., collects at these ends and they are favourite places for mosquitoes breeding.

4. Fish must be kept in the main gudwals, and the latter must be re-stocked with fish after emptying and filling.

Branch gudwals

5. The beds of branch gudwals must, if possible, be allowed to dry before fresh water is run on to them. If this is impossible it is essential that all pools in these beds be abolished before the channels are refilled.

6. All inlets to irrigated land must be properly closed when irrigation is not proceeding.

7. Grass growing at the bottom of gudwals must be kept as short as possible and, where it is feasible, should be rooted out.

8. Particular attention must be paid to any branch gudwal which is above the ground level and hence has a tendency to leak.

9. Wherever the incoming water tends to scoop out the bed of a gudwal, *i.e.* at the entrance to branch gudwals, a bed of stones should be laid down.

Cultivated areas

10. Care should be taken to see that there are no areas of depression in which water will accumulate and form pools. This is especially important in the case of land on which crops like wheat, barley and sugar-cane are grown, as the thick growth conceals these places and prevents evaporation.

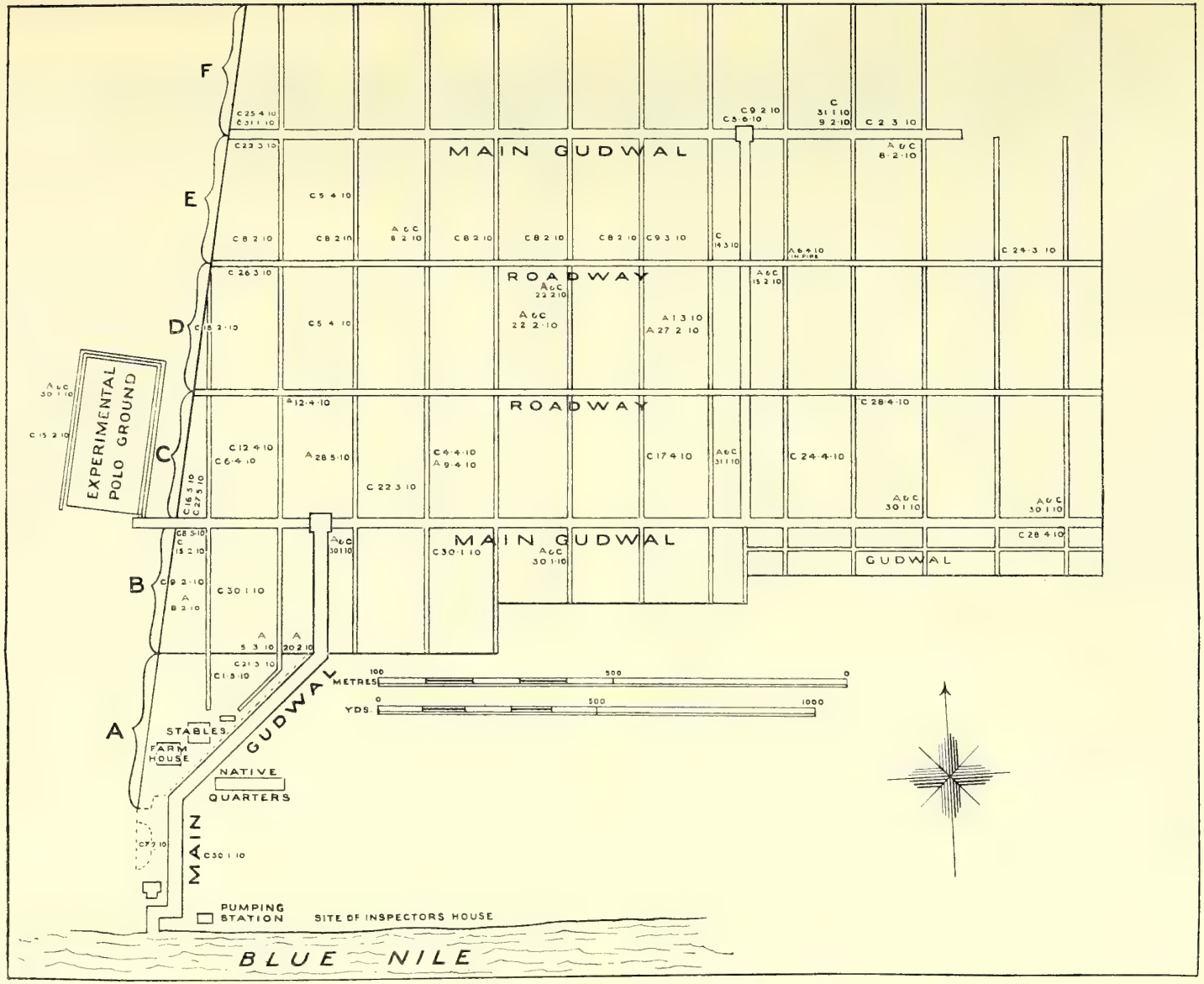
Leakages in the banks of channels

It is to be remembered that the soil at the farm in question is what is called "weeping," *i.e.* contains much salt. This is apt to be dissolved out, and hence leakages in the earthen banks of channels are frequent. At one time I thought I would have to press for the total or partial abolition of this irrigated area, but since the regulations have been issued and those responsible have been got to take an intelligent view of the situation and to co-operate heartily with the Sanitary Service, I am bound to say that things have greatly improved and, at present, and for some time past, the farm has been well-nigh free of all infection. It is hoped this happy state of affairs may persist,[1] though I fear we must anticipate more trouble in the summer when a larger quantity of water is available for irrigation and when heavy rains may complicate matters.

While on this subject I may quote a short but instructive paper by Dr. Gordon, of Messina, N. Transvaal, who comments upon a previous article by Dr. Spencer, of Middelburg. He says: "A valuable lesson to be drawn from Dr. Spencer's article in the February issue of the *Transvaal Medical Journal*, lies in the illustration it affords of the fact that the term 'cultivation of the soil' must be interpreted in the sense of scientific agriculture, if cultivation is to be acknowledged as an eradicator of the anopheline.

"It has been abundantly proved in other countries that malaria disappears before the advance of scientific agriculture. Dr. Spencer shows that 'cultivation,' as practised in the districts with which he is familiar, results in the *increase* of malaria. It is therefore

[1] It has done so up to the present—end of April, 1911.—A.B.



W. H. McLean

FIG. 88.—PLAN OF SUDAN DEVELOPMENT AND EXPLORATION COMPANY'S FARM

Showing mosquito breeding-places from January 30, 1910, to June 5, 1910

A = Anophelines C = Culex

justifiable to conclude that the methods of cultivation employed in those districts are crude and unscientific.

"A principle of scientific cultivation is the preservation of subsoil moisture by the prevention of surface evaporation. The method necessitates the regular use of the cultivator or acme-harrow after rain or irrigation so that the land is left covered with a thick and even mulch of loose soil.

"Dr. Spencer speaks of holes and irregularities present in 'cultivated' soil. There are no such breeding-places of anopheles in land which has been cultivated scientifically; the beautifully even mulch abolishes them. If the agriculture of the districts referred to were conducted on scientific lines it would not have been possible for Dr. Spencer to trace so ably a distinct connection between the incidence of malaria and the vicinity of 'cultivated' land.

"A second lesson is made evident by these facts. With the extension of maize and fruit growing, it is possible that irrigation will be introduced into those dry districts where malaria has not prevailed, solely owing to the lack of breeding-places for anopheles.

Necessity for drainage

Beginners in the practice of irrigation—I speak out of past experience—are apt to forget that such crops as maize and fruit under irrigation require also adequate drainage. Stagnant pools and even a water-logged soil may eventually be caused by irrigation when proper cultivation is not carried out or when drainage is not provided: especially if, as is frequently the case, the irrigation shows *trop de zèle* on the part of the farmer. Thus the anopheline may conceivably be introduced into new districts in the wake of extended agriculture. Where the nature of the crop—as in the case of sugar-cane—requires the permanent presence of flumes, trenches and canals, efficient anti-malaria measures are necessary, such as attention to the construction of the canals, careful removal of the weeds, frequent flushing and stocking with fish which consume the larvæ and pupæ and eggs of anopheles.

"The conclusion may be suggested that education in the suitable methods of malaria-prevention should go hand-in-hand with education in scientific agriculture in this country."

With these statements I am in complete accord, and the problem is more difficult in the Sudan solely because hitherto many of our farmers have been amateurs rather than experts, and were, perhaps, better at raising mosquitoes for the annoyance and to the danger of their fellows, than the crops required for their sustenance and benefit.

Influence of Cultivation on Other Diseases

Ankylostomiasis and bilharziosis

In our First Review Supplement attention was directed to the danger of both ankylostomiasis and bilharziosis increasing in the Sudan as more and more land was brought under cultivation by irrigation methods and as Egyptian fellaheen entered the country in greater numbers to play the rôle of cultivators. The matter was brought before the Central Economic Board and, so far as medical inspection of immigrants can check the danger, it is likely that all will be well, but the risk to individual health and national prosperity from such operations, on a large scale, should ever be borne in mind, although, just as in the case of anti-malarial measures in their connection with agriculture, it must ever be a question of "Is the game worth the candle?"

As regards the mosquito work, generally, one need not say much. We are about to test the emulsion-forming larvicide introduced for the great anti-malarial work at Panama.

It will be used in river pools and other likely breeding-places of this type. A point noted by Mr. Murray, our chief Sanitary Inspector, and worthy of mention, is, that pools with clay bottoms are much more favoured by anophelines than those with sandy beds.



G. Buchanan

Fig. 89.—Irrigation Canal, Sudan Development and Exploration Co.'s Farm



G. Buchanan

Fig. 90.—Irrigation Canal, Sudan Development and Exploration Co.'s Farm

Clay pools more often affected by anophelines than sand pools

This is no doubt due to the fact that the former are more permanent and the water in them clearer. Probably also they yield a more abundant food-supply for the larvæ. A practical point also is the knowledge that well-oiled rags wrapped round the legs of tables bearing foodstuffs will keep off ants, thereby forming excellent substitutes for the water-containing vessels mentioned in the list of breeding-places.

While there are, I am glad to say, many persons in Khartoum public spirited enough to aid the sanitary service, and in this connection I would specially mention Mr. F. S. Sillitoe, who has charge of all Government gardens, there is also, amongst officials and others, a great deal of lethargy and carelessness. Like Gallio, they care for none of these things. Fortunately they can be fined and are fined, but it is a painful fact that some would rather be fined, or let their servants be fined, than take the trouble to inspect their own zeers and other water collections on their premises. It is fortunate for themselves that such individuals do not live in New Orleans or Panama where they have a short and effective way of dealing with those who endanger the public health or comfort.

Perhaps if these individuals reflected that several hundreds of imported cases of malaria must yearly reach Khartoum, the majority of which are able to serve as distributing centres of the disease, that infected anophelines are brought now and then into the town by boats and steamers and are attracted by likely breeding-places, that *Stegomyia calopus* can very probably convey the virus of horse-sickness and of dengue and that *Culex fatigans* is almost certainly another carrier of dengue fever, they might be more willing to devote a small portion of their time to aiding the mosquito brigade.

Proof of their carelessness has recently been forthcoming, for the substitution of clear deep-well water for muddy river water has done away with the necessity, from the household standpoint, of frequently cleaning out zeers. As a result, the servants have neglected this work and the masters have too often acquiesced in this neglect. As a result, *Stegomyia* has been finding breeding-places in the town. As one despairs of ever getting certain of these defaulters into line for, as Solomon says, one may bray a fool in a mortar without benefit resulting, it has now, with the consent of the Governor, been resolved to issue an order to the effect that all zeers must be supplied with a cover of a special type (Fig. 92).

Zeers to be fitted with a special cover

The weighing material is sand, suggested by Dr. Beam, and it is both cheap and effective. A tape can also be used, passed through the edge of the cover, and pulled tight and fastened as required.

Here is the type of notice circulated, a point of practical interest:—

"Owing to the fact that clear well water is now being supplied instead of the river water previously in use, it is not, from the point of view of mere convenience, so necessary to clean zeers frequently. Servants are now, therefore, neglecting to carry out this duty and, as in the majority of cases, their masters, despite many appeals and warnings from the Sanitary Service, will not exercise the necessary personal supervision, it has become imperative to take more stringent measures. Every zeer, barrel and similar receptacle containing water in Khartoum should be kept covered by a suitable cover for the purpose of preventing mosquitoes gaining access to such water collections. Covers of an approved pattern as recommended for use can be seen at the office of the Chief Sanitary Inspector at the Mudiria. Anyone found harbouring mosquito-larvæ, of any size or age, in water receptacles which can be fitted with the above-mentioned covers will be prosecuted."

Figs. 91 and 93 show how all zeers and burmas in native villages and localities must be above ground level and not sunk in the soil as heretofore. It is now but rarely that we find this order contravened.



G. Buchanan

Fig. 91.—Lines of Zeers on Stands in Town Quarter, Khartoum North



G. Buchanan

Fig. 92.—Zeer with Cover



G. Buchanan

Fig. 93.—Village Street showing Burmas on Stands

Other sanitary matters call for little notice here, as the whole question of conservancy was gone into very fully in our Third Report, and water-supply receives a special chapter to itself, but one would record a great extension of the small incinerator system, the cheap and effective type now in use being shown in Fig. 81 (*page* 259).

Rats in Khartoum

Thanks to the kindness of Captain S. S. Flower, of Cairo, I am able to submit a list of the species of rats existing in Khartoum. Out of twenty specimens sent, Captain Flower, whose determinations were kindly confirmed by Mr. J. L. Bonhote, recognised ten as *Arvicanthis testicularis*, a species of garden rat, eight as *Mus norvegicus*, the ship or Norway rat, and two as *Mus rattus*, the black rat, and the principal plague carrier amongst rodents, at least in India. It is probable that other species occur, but, owing to pressure of other work, I have been unable to carry out further investigations in this direction.

We have abandoned the use of ratin, and now employ, along with traps, a very useful phosphorous paste which is easily handled and very deadly. So far the rats have not learned to avoid it. The Sakias on the river banks appear to be the favourite homes of these destructive animals, which a special brigade of four men is doing its best to reduce in numbers.

Prevalence of Infectious Diseases

The last statistics given were those for ten months of 1907–8.

Return of infectious diseases

One can now give the complete statistics for that sanitary year and also those for 1908–9 and 1909–10. It may be said at once that imported cases of malaria need not be reported. The few sent in are, however, always entered in the books. As before, one must state that too much reliance cannot be placed on these figures, but every year the returns are becoming more reliable, and there is no doubt that the figures afford a very fair indication of the amount of infectious disease present in the town.

NOTIFIABLE DISEASES REPORTED DURING THE YEAR FROM OCTOBER 1, 1907, TO SEPTEMBER 30, 1908

Disease	Civil	Egyptian Military	British Barracks	Imported	Total
Cerebro-Spinal Fever	3	—	—	—	3
Chicken-pox	36	1	—	—	37
Diphtheria	2	—	—	—	2
Dysentery	28	17	1	4	46
Enteric	16	6	—	1	22
Erysipelas	3	—	—	—	3
Kala-azar	—	2	—	2	2
Leprosy	—	1	—	1	1
Malaria	1	1	—	—	2
Measles	1	—	—	—	1
Whooping Cough	1	—	—	—	1
Total	91	28	1	8	120

The other notifiable diseases were not represented.

TABLE OF MONTHLY INCIDENCE

Disease	1907			1908								
	October	November	December	January	February	March	April	May	June	July	August	September
Cerebro-Spinal Fever ...	—	—	—	—	—	—	—	2	1	—	—	—
Diphtheria...	2	—	—	—	—	—	—	—	—	—	—	—
Dysentery	2	1	6	2	4	3	—	6	3	6	12	1
Enteric	—	2	1	1	—	—	—	2	5	3	4	4
Malaria	—	—	—	1	—	—	1	—	—	—	—	—

SPECIAL TABLE

		European	Native
Enteric Fever ...	Cases	14	8
	Approximate percentage calculated on population ...	0·35	0·016
Dysentery	Cases	12	35
	Approximate percentage calculated on population ...	0·3	0·068

NOTIFIABLE DISEASES REPORTED DURING THE YEAR FROM OCTOBER 1, 1908, TO SEPTEMBER 30, 1909

Disease	Civil	Egyptian Military	British Barracks	Imported	Total Local Cases
Chicken-pox	43	7	—	3	50
Diphtheria	1	1	—	—	2
Dysentery	25	20	5	3	50
Enteric	11	5	—	3	16
Erysipelas	1	—	1	—	2
Kala-azar	—	—	—	7	—
Leprosy	—	—	—	1	—
Malaria	1	4	2	—	7
Measles	2	—	—	2	2
Total	84	37	8	19	129

The other notifiable diseases were not represented.

TABLE OF MONTHLY INCIDENCE

Disease	1908			1909								
	October	November	December	January	February	March	April	May	June	July	August	September
Diphtheria	—	—	—	—	—	—	1	—	—	—	—	1
Dysentery	8	8	8	3	1	3	—	2	3	2	10	2
Enteric	1	3	2	—	2	2	—	3	—	3	—	—
Malaria	—	4	2	—	—	—	—	1	—	—	—	—

SPECIAL TABLE

		European	Native
Enteric Fever ...	Cases	10	6
	Approximate percentage calculated on population ...	0·25	0·012
Dysentery	Cases	8	42
	Approximate percentage calculated on population ...	0·20	0·084

The tables for 1909–10 are a little more elaborate, and it must be noted that the population has increased, now numbering about 61,392.

NOTIFIABLE DISEASES REPORTED DURING THE YEAR FROM OCTOBER 1, 1909, TO SEPTEMBER 30, 1910

Disease	Civil	Egyptian Army	British Barracks	Imported	Local Cases	Totals
Blackwater Fever	—	—	—	1	—	1
Cerebro-Spinal Meningitis	1	—	—	—	1	1
Chicken-pox	15	—	—	3	15	18
Diphtheria	3	2	—	1	5	6
Dysentery	7	2	6	6	15	21
Enteric Fever...	4	3	2	4	9	13
Erysipelas	1	1	2	—	42	4
Kala-azar	—	—	—	4	—	4
Malaria...	7	3	9	41	19	60
Malta Fever	1	1	—	1	2	3
Measles...	1	—	—	3	1	4
Relapsing Fever	—	2	—	3	2	5
	40	14	19	67	113	140

The other notifiable diseases were not represented. As a matter of interest, I present the above table in another form, showing the monthly incidence and local distribution of the reported cases.

		October	November	December	January	February	March	April	May	June	July	August	September	Totals
Blackwater Fever	K	—	—	—	—	—	—	—	—	—	—	—	—	—
	N	—	—	—	—	—	—	—	—	—	—	—	—	—
	I	—	—	—	—	—	—	—	—	1	—	—	—	1
Cerebro-Spinal Meningitis	K	1	—	—	—	—	—	—	—	—	—	—	—	1
	N	—	—	—	—	—	—	—	—	—	—	—	—	—
	I	—	—	—	—	—	—	—	—	—	—	—	—	—
Chicken-pox	K	—	—	1	—	—	—	6	4	2	1	—	—	14
	N	—	—	—	—	—	1	—	—	—	—	—	—	1
	I	—	—	—	—	—	—	3	—	—	—	—	—	3
Diphtheria	K	—	—	1	1	—	2	—	—	—	—	—	—	4
	N	1	—	—	—	—	—	—	—	—	—	—	—	1
	I	—	—	—	—	—	1	—	—	—	—	—	—	1
Dysentery	K	—	—	5	2	—	—	—	—	—	—	—	2	9
	N	—	—	—	—	—	—	—	—	—	—	1	5	6
	I	—	—	1	1	—	—	1	—	2	1	—	—	6
Enteric Fever	K	2	—	—	1	—	—	—	—	—	1	—	1	5
	N	—	—	1	—	—	—	—	—	—	1	1	1	4
	I	—	1	—	—	1	—	—	1	—	1	—	—	4
Erysipelas	K	—	—	—	—	1	—	—	—	—	—	—	—	1
	N	—	—	—	—	—	—	1	—	—	—	—	—	1
	I	—	—	—	—	—	—	—	—	—	—	—	—	—
Kala-azar	K	—	—	—	—	—	—	—	—	—	—	—	—	—
	N	—	—	—	—	—	—	—	—	—	—	—	—	—
	I	—	1	—	2	—	—	—	—	—	—	—	1	4
Malaria	K	—	—	4	4	2	6	—	1	—	—	—	—	17
	N	—	—	—	1	—	—	—	1	—	—	—	—	2
	I	—	1	5	4	3	10	2	4	1	10	1	—	41
Malta Fever	K	—	1	—	—	—	—	—	—	1	—	—	—	2
	N	—	—	—	—	—	—	—	—	—	—	—	—	—
	I	—	—	—	—	—	—	—	1	—	—	—	—	1
Measles	K	—	—	—	—	—	—	—	—	—	—	—	—	—
	N	—	—	—	—	—	—	—	—	1	—	—	—	1
	I	—	—	—	—	1	—	—	—	1	—	1	—	3
Relapsing Fever	K	—	—	—	1	—	—	—	—	—	—	—	—	1
	N	—	—	—	—	—	—	—	—	—	—	—	—	—
	I	—	1	—	3	—	—	—	—	—	—	—	—	4

K = Khartoum N = Khartoum North I = Imported

As previously, I also give a small table showing, separately, the monthly incidence of four of the more important of the above diseases :—

Disease	October	November	December	January	February	March	April	May	June	July	August	September
Diphtheria	1	—	1	1	—	2	—	—	—	—	—	—
Dysentery	—	—	5	2	—	—	—	—	—	—	1	7
Enteric Fever	2	—	1	1	—	—	—	—	—	2	1	2
Malaria	—	—	4	5	2	6	—	2	—	—	—	—

and, finally, a new type of table exhibiting the incidence of enteric fever and dysentery respectively upon the European and the Native population and upon the military and civil communities.

ENTERIC FEVER FROM OCTOBER 1, 1909, TO SEPTEMBER 30, 1910

Month	Khartoum						Khartoum North						Imported	Total
	Europeans			Natives			Europeans			Natives				
	Military	Civil	Total	Military	Civil	Total	Military	Civil	Total	Military	Civil	Total		
October	1	—	1	—	1	1	—	—	—	—	—	—	—	2
November	—	—	—	—	—	—	—	—	—	—	—	—	1	1
December	—	—	—	—	—	—	1	—	1	—	—	—	—	1
January	1	—	1	—	—	—	—	—	—	—	—	—	—	1
February	—	—	—	—	—	—	—	—	—	—	—	—	1	1
March	—	—	—	—	—	—	—	—	—	—	—	—	—	—
April	—	—	—	—	—	—	—	—	—	—	—	—	—	—
May	—	—	—	—	—	—	—	—	—	—	—	—	1	1
June	—	—	—	—	—	—	—	—	—	—	—	—	—	—
July	1	—	1	—	—	—	—	1	1	—	—	—	1	3
August	—	—	—	—	—	—	—	—	—	—	1	1	—	1
September	—	1	1	—	—	—	1	—	1	—	—	—	—	2
	3	1	4	—	1	1	2	1	3	—	1	1	4	13

CASES OF DYSENTERY FROM OCTOBER 1, 1909, TO SEPTEMBER 30, 1910

Month	Khartoum						Khartoum North						Imported	Total
	Europeans			Natives			Europeans			Natives				
	Military	Civil	Total	Military	Civil	Total	Military	Civil	Total	Military	Civil	Total		
October	—	—	—	—	—	—	—	—	—	—	—	—	—	—
November	—	—	—	—	—	—	—	—	—	—	—	—	—	—
December	5	—	5	—	—	—	—	—	—	—	—	—	1	6
January	2	—	2	—	—	—	—	—	—	—	—	—	1	3
February	—	—	—	—	—	—	—	—	—	—	—	—	—	—
March	—	—	—	—	—	—	—	—	—	—	—	—	—	—
April	—	—	—	—	—	—	—	—	—	—	—	—	1	1
May	—	—	—	—	—	—	—	—	—	—	—	—	—	—
June	—	—	—	—	—	—	—	—	—	—	—	—	2	2
July	—	—	—	—	—	—	—	—	—	—	—	—	1	1
August	—	—	—	—	—	—	—	—	—	1	—	1	—	1
September	1	—	1	—	1	1	—	1	1	—	4	4	—	7
	8	—	8	—	1	1	—	1	1	1	4	5	6	21

It will be at once apparent that the amount of enteric fever has been trifling. Doubtless this is in part due to a favourable year so far as climatic conditions go. Although during the summer months the usual high temperatures obtained, the rainfall was less than usual and there was a notable diminution in the number of severe dust storms. At the same time, one must not overlook the fact that the town, with the exception of Khartoum North, has been in possession of a pure water-supply, albeit up to the present the distribution of the water in certain localities has left a great deal to be desired. One cannot, of course, expect to draw any conclusions from such small figures, but some may find it interesting to speculate upon them, and for such I would contrast the marked diminution in the dysentery returns with the establishment of many more small incinerators which now serve the native villages to the south of the town, and serve them well.

Diminution in dysentery

Last year there were 50 cases locally acquired, this year there have only been 15, and one knows for certain that two of these were not true dysentery but were induced by faulty dietetic habits. (Evidence of Senior Medical Officer, British Troops.) Other causes which may in some measure have aided the reduction of these two diseases are an improvement in scavenging arrangements, the treatment of zibla heaps to prevent flies breeding out in them, and a more careful cleansing of the conservancy buckets.

Moreover, we are now at pains, so far as dysentery is concerned, to distinguish imported and relapse cases and to subtract them from the total, as such cases are not, as a rule, due to local faulty conditions. At least we can say that the local conditions were not favourable to the spread of either of these diseases.

The malaria cases have already been commented upon, and the number is a matter for regret but may perhaps serve a useful purpose as a warning and reminder. Happily none of the cases proved fatal. It is, of course, practically impossible to prevent cases due to infected anophelines being brought into the town by steamers or boats. Such cases are happily rare, but they do occur and increase our returns.

As regards other diseases, it will be noticed that the spread of cerebro-spinal fever, measles and diphtheria was checked—an interesting example I hope of what can be done by modern sanitary methods. Relapsing fever figures for the first time. The original cases came from Egypt and the locally-acquired cases apparently derived infection from clothes lice. The small epidemic was studied in the laboratories and some evidence advanced to show that the spirochæte of Egyptian relapsing fever is a specific entity. This is confirmatory of work done in Egypt and Algiers. In the latter country the causal spirochæte is recognised as a new species and named *Sp. berbera*. It is possibly the etiological factor in all the spirochætosis epidemics of Northern Africa.

Relapsing fever recorded in the Sudan for the first time

Two cases of Malta fever, apparently locally acquired, fall to be recorded. In one the source of infection could not be traced, but the diagnosis was confirmed by laboratory methods. The other was a case of laboratory infection *(vide page* 220*)*.

Fortunately, there is no evidence that, apart from imported cases, kala-azar exists in Khartoum. Until we are certain of the vector in this disease it will not be possible to account for this apparent immunity.

Phlebotomus fever, carried by the so-called "sand-fly," really a moth fly, the tiny irritating insect which haunts verandahs, has been made notifiable. In all probability it occurs at certain seasons.

Vital Statistics

Thanks to the Assistant Director, Sudan Medical Department, I am able for the first time in the history of the town to present some figures regarding the births and deaths which have occurred amongst the civil population. So far as the deaths are concerned,

these, as Dr. Crispin points out, cannot be taken as absolutely reliable and in this respect differ from those kindly furnished by the Senior Medical Officer, Khartoum District, for the native military population, and by the Senior Medical Officer, British troops, for the men under his charge.

Crude death-rate

On the basis of these figures I have worked out a crude death-rate which, however, furnishes little of special value. I have found it impossible, owing to lack of reliable information, to present a recorded death-rate, and still less a corrected death-rate. In the former, allowance is made for the deaths of persons in public institutions, and it is intended to exclude the deaths of persons who, though not inhabitants, happen to die in the town. The latter is a recorded death-rate corrected for the age and sex distribution of the population, and, naturally, in a place like Khartoum, it is hopeless at present to think of attaining such statistical accuracy.

During the sanitary year, 1909–10, there occurred amongst the civil population of Khartoum, including persons resident in the daims, in the villages at Buri, and the villages at the Point, 414 births and 290 deaths.

The figures for Khartoum North are 195 and 116, respectively. In the Egyptian Military Hospital there were 10 deaths amongst the Khartoum garrison (about 3000 men), while amongst the British troops (about 800 men) there were 6, four of which were due to drowning. Putting these into tabular form we find:—

	Births	Deaths
Civil population, Khartoum	414	290
Civil population, Khartoum North	195	116
Egyptian Army (Khartoum and Khartoum North)	—	10
British troops	—	6
Total	609	422

Working this out on a population of 60,000—a fair calculation, and one making ample allowance for any possible over-estimate—we obtain a crude death-rate of a little over 7 per 1000, that is to say, one death in every 142 persons. This would be a very gratifying figure, especially as it is a crude death-rate, if one could rely absolutely upon it, but it is necessary to take it with the proverbial grain of salt. Still, all things considered, for burial is not permitted unless a certificate is forthcoming, it cannot be very far wrong, and gives a general indication that the death-rate of the city is distinctly low. It will be interesting to see how this figure will compare with those of subsequent years when statistics will be more reliable and complete, and it is more for this reason than for any other that I record it.

Difficulty in arriving at the birth-rate

The crude birth-rate based on the returns is only a little over 10 per 1000, and is obviously erroneous, though it has to be remembered that a considerable proportion of the population is military, official, and unmarried, and that there are also many unmarried European and, especially native, labourers who come into the town for work, leaving their wives and families behind them.

Deaths are much more likely to be reported properly than births, and in any case a birth-rate, to be of comparative value, should be calculated on the number of annual births per 1000 women living at child-bearing ages.

Thanks to the courtesy of Dr. Crispin and of the Medical Officer, Khartoum North,

and to the kind help of Mr. John Newlove, one is able to classify the births and deaths in certain useful and suggestive tables.

In the first place, one presents a monthly table of incidence for Khartoum only, which does not seem to prove very much one way or the other, but may be useful for future comparison and shows anyhow that malaria has no influence on the death returns.

No. OF BIRTHS AND DEATHS IN KHARTOUM (CIVIL ONLY) FROM OCTOBER 1, 1909, TO SEPTEMBER 30, 1910

Month	Births	Deaths
October, 1909	36	26
November, 1909	28	24
December, 1909	32	21
January, 1910	37	24
February, 1910	39	10
March, 1910	31	23
April, 1910	33	24
May, 1910	40	27
June, 1910	35	26
July, 1910	31	28
August, 1910	35	26
September, 1910	37	26
	414	285 + 5 (undifferentiated) = 290

The second table classifies the deaths according to nationality and age, and I do not intend to comment upon it beyond pointing out the gratifying fact that only one person of British nationality amongst the civil population died during the year.

Nationality and Approximate Ages of Recorded Deaths; September, 1909, to September, 1910:—

Nationality	Under 2		Over 2-12		Adults		Total	
	M.	F.	M.	F.	M.	F.	M.	F.
Abyssinian	—	—	—	—	4	2	4	2
British	—	—	—	—	1	—	1	—
Egyptian	13	16	—	—	4	8	17	24
Greek	2	—	—	—	3	1	5	1
Italian	—	1	—	—	1	1	1	2
Sudanese	19	18	10	6	92	83	121	107
Syrian	—	1	1	—	1	1	2	2
Turk	—	—	—	—	—	1	—	1
	34	36	11	6	106	97	151	139

A somewhat similar but less detailed table for Khartoum North is next shown:—

Births	Deaths							
Total	Total	Males	Females	Natives	Egyptians	Europeans	Infants	Under puberty
195	116	52	64	102	13	1	23	3

In neither table is any attempt made to exclude the deaths of non-residents.

The second table can only be taken as approximately correct, but shows only one death amongst Europeans there. Finally, we consider the causes of death, obtained from the four sources already mentioned. They are arranged merely in alphabetical sequence.

1. BRITISH TROOPS

Deaths	
Cause	Number
Gastro-Enteritis	2
Suffocation from submersion	4
Total	6

I understand both the cases of gastro-enteritis were probably due to ptomaine poisoning.

2. EGYPTIAN MILITARY

Deaths	
Cause	Number
Appendicitis	1
Bronchitis, Jaundice	1
Cerebral abscess	1
Dysentery	1
Gastric ulcer	1
Nephritis	1
Osteomyelitis of femur	1
Pneumonia	1
Purpura	1
Suffocation from submersion	1
Total	10

3. CIVIL, KHARTOUM

Cause of Death	Under 2	2–12 years	Adults
Acute Gastro-Enteritis	2	—	2
Blackwater Fever	—	—	1 (Bahr-El-Ghazal)
Bronchitis	1	2	1
Cancer	—	—	3
Cerebral Hæmorrhage	—	—	6
Chronic Nephritis	—	—	1
Concussion of Brain	—	—	1
Continuous Fever	11	1	6
Debility	4	—	2
Diarrhœa	8	—	14
Enteric Fever	—	—	1
Enteritis	1	—	1
Epilepsy	—	—	2
Fever	5	2	5
Fever and Diarrhœa	—	—	1
Heart Disease	—	—	9
Hemiplegia	—	—	6
Immaturity at Birth	7	—	—
Intermittent Fever	3	—	2
Jaundice	—	—	1
Marasmus	3	—	—
Meningitis	1	1	1
Pneumonia	3	—	12
Puerperal Fever	—	—	2
Septicæmia	1	1	1
Sunstroke	—	—	1
Tetanus	—	—	2
Tubercular Enteritis	—	—	1
Tubercular Meningitis	—	1	—
Tuberculosis	1	—	55
Ulcers	1	—	1
	52	8	141

This table should be combined, and read in alphabetical sequence, with that on page 285

3. CIVIL, KHARTOUM—*continued*

Cause of Death	Under 2	2–12 years	Adults
Brought forward	52	8	141
Ascites	1	1	9
Burns	—	—	1
Chronic Hepatitis	—	1	1
Cystitis	—	—	1
Diabetes	—	—	1
Difficult Labour	—	—	2
Diphtheria...	1	—	—
Drowning	—	—	2
Dysentery	6	3	11
Endocarditis	—	—	1
Fracture of Spine	—	—	1
Gangrene	1	—	4
Gastritis	1	—	1
Hernia	—	—	2
Intestinal Obstruction	—	—	1
Intussusception of Intestines	—	—	1
Laryngitis	—	1	—
Liver Abscess	—	—	1
Madura Foot	—	—	1
Necrosis	—	—	1
Pelvic Abscess	—	—	1
Periostitis	1	—	1
Peritonitis	—	—	5
Pharynx, contused wound of	—	—	1
Retention of Urine	1	—	2
Sarcoma	—	—	1
Scorpion Sting	1	2	—
Senile Decay	—	—	5
Syphilis	—	—	2
Syphilis, hereditary	5	1	—
Tubercular Glands	—	—	1
Urethral Constriction	—	—	1
	70	17	203

This table should be combined, and read in alphabetical sequence, with that on page 284

I understand from Dr. Crispin that a certain number of these cases, especially those in outlying villages, were diagnosed by native sanitary barbers, whose instructions are to record the most prominent symptom at the time of death. This explains the inclusion of such terms as "diarrhœa" and "fever" which are symptoms, not diseases. In view of the notification returns for communicable diseases, the presence of 20 fatal cases of dysentery provides room for reflection, but one knows that there is no class of disease in the Sudan in which so many mistakes in diagnosis are made as that in which blood in the stools is a prominent symptom.

Various conditions returned as Dysentery

I have seen hæmorrhoids, tubercular ulceration of the bowel, simple enteritis, bilharzia of the rectum and several other conditions, returned as dysentery, and it is probable that not one-half of the cases under discussion were either true bacillary or amœbic dysentery. Moreover, several of them may have been imported or relapse cases. At the same time, one cannot but admit that this table with its entries of diarrhœa,[1] fever and dysentery, throws some doubt on the official dysentery returns, and that there were, probably, as indeed I have indicated, considerably more dysentery cases than were notified. Even so, however, there is no reason to alter the statement made on *page* 280. The official notifications from year to year give, beyond any doubt, a fair idea of the amount of communicable disease present in the town and certainly the death returns indicate that the health of the community is at present satisfactory and will, I trust, lead to an increased support of those sanitary measures on which so much depends. One has no wish to claim too much for sanitation, and we must remember the character of the population, the climate, the comparative isolation of the city and everything having a bearing on the question. The very presence, however, of diseases like malaria, dysentery and enteric surely indicates that, given faulty conditions, they will speedily increase and that it is essential to combat them in every possible way. Water-supply, milk-supply, improper food, disposal of excreta, waste waters and refuse; mosquitoes, flies and dust, all demand careful attention, as do possible carrier cases and imperfect housing conditions.

Liability of the Sudanese to Tuberculosis

As regards the last point, observe the part tuberculosis plays in the table, accounting directly for 58 deaths and indirectly probably for many more, as there can be little doubt that some of the cases of so-called diarrhœa, fever and dysentery were in reality tuberculous in nature. The Sudanese are specially susceptible to the bacillus of tubercle and time and again I have seen obscure febrile cases turn out to be general tuberculosis. I believe the prevalence of this disease is due in large measure to the exclusion of light and air from their primitive and over-crowded dwellings, to their filthy habit of expectoration, and to some extent also to the nature of their dietary. It is, perhaps, noteworthy that nearly all the deaths occurred amongst adults, some proof of the accuracy of the hypothesis advanced, for tuberculosis in children is believed to be largely due to infection through the intestinal tract.

The last table is that for Khartoum North. It is very unsatisfactory from a statistical standpoint and hardly worth including.

Of the 116 deaths, it will be seen that only 30 have the cause of death stated. The remaining 86 have been returned as either intestinal diseases or fevers—terms which, under the conditions obtaining at Khartoum North, mean next to nothing. The medical officer reports that they were not seen until after death had occurred. By presenting such a table, however, and drawing attention to the great value of reliable statistics and the need for improvement a useful purpose is served. To me the tables

[1] In this connection, see a very able and suggestive paper by Dr. G. D. Maynard, of Pretoria, entitled "Aestival Diarrhœas of South Africa." *Transvaal Medical Journal*, September, 1910.

KHARTOUM NORTH

Cause of Death	Number of Deaths
Apoplexy	2
Asphyxia (accidental)	1
Cirrhosis of liver	1
Diphtheria	1
Heart disease	3
Hereditary syphilis	1
Judicial hanging	1
Lightning stroke	1
Malarial Fever	4
Phthisis	2
Pneumonia	3
Puerperal Fever	3
Scorpion bite	2
Suffocation from submersion	4
Violence (murder)	1
Total	30

have been both interesting and instructive, and I cannot but think they will prove so to those who are interested in the welfare of the city and in the sanitary measures taken to maintain and improve its public health. The next step should, I think, be in the direction of checking both the spread of tuberculosis and those venereal diseases which are, unfortunately, so common and tend so greatly to sap the strength and procreative power of a community.

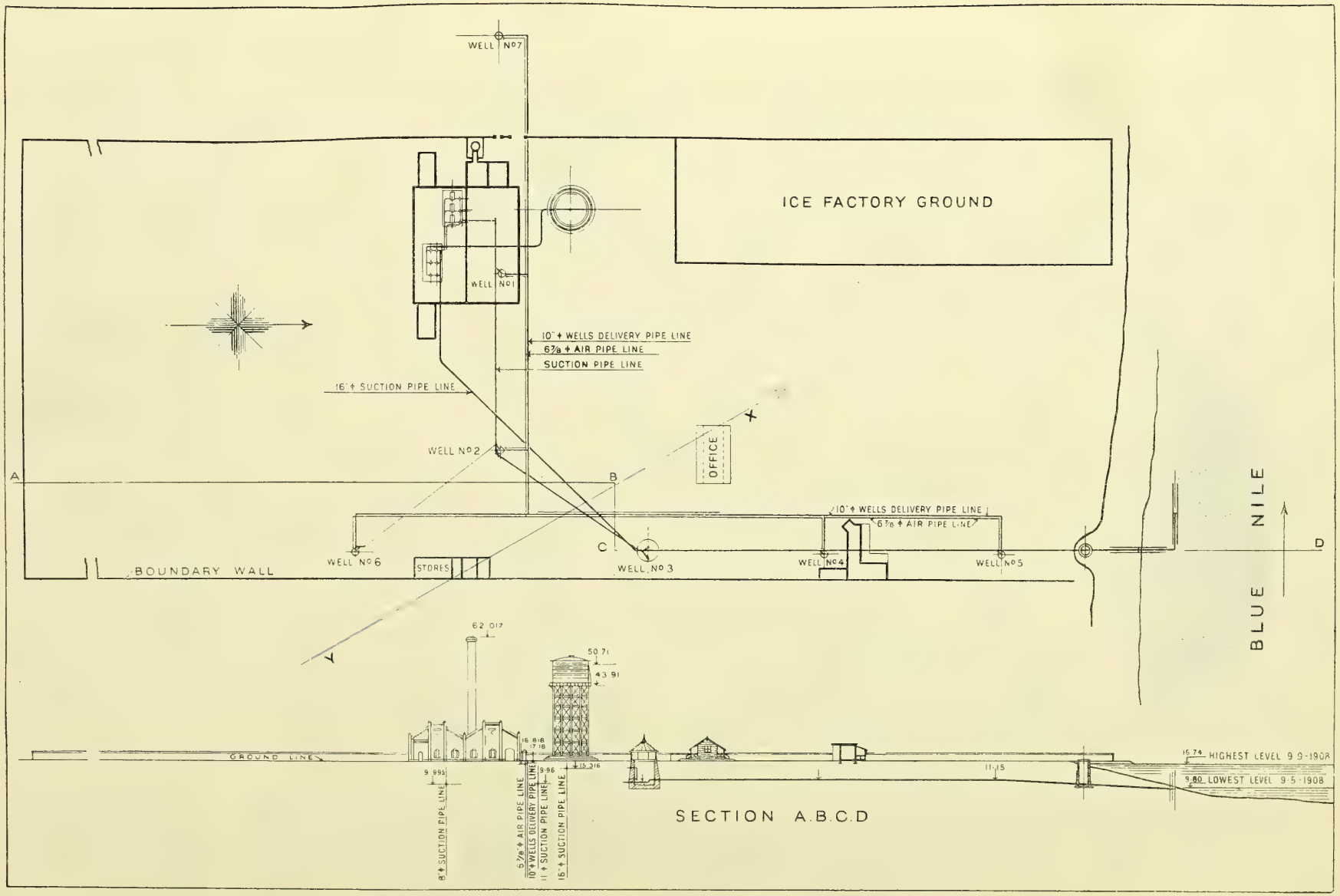


FIG. 94.—SITE, PLAN AND ELEVATION OF BURI WATER WORKS, KHARTOUM

Wells Nos. 2, 3, 4 and 5 are no longer in use. A large covered reservoir now occupies a site near the old "office"

From plan prepared by Mr. Williams and reproduced with the kind permission of the Director of Works

The Water-Supply of Towns in the Tropics Chiefly from the Bacteriological Standpoint as Illustrated by the Water-Supply of Khartoum

BY

The Director

As events are trending there may yet, in certain instances at least, be some justification for those who express a lack of faith in the value of *Bacillus coli communis* as an indicator of pollution of a potable water. This organism has been put forward by Houston, Savage, Jordan and others[1] as, with due restrictions, a reliable test of purity or otherwise of a water-supply. Their view has recently received confirmation at the hands of Portuguese investigators, by Guiraud and Mandoul[2] in France, and has also been ably upheld by Fromme[3] in an exhaustive paper in the *Zeitschrift für Hygiene*, the result of a lengthy experience and many investigations. Similar conclusions were reached by Hilgermann.[4] On the other hand there seems to be a growing tendency to regard it as mistaken, and I need merely mention recent expressions of opinion by Starkey[5] and Russel and McLean[6] in this connection, while the majority of German workers consider the bacillus ubiquitous. In this connection a recent paper by Gärtner[7] may be consulted. One has neither the time nor the desire to plunge into this vexed question so far as water-supplies generally are concerned, but would direct attention to it as regards water-supplies in the Tropics. Until recently, records of bacteriological investigations of water-supplies in tropical countries have been few and far between. This is natural enough as, hitherto, facilities have not existed for work on those lines, but with the establishment of well-equipped laboratories in many parts of the tropical world it is probable that ere long many statistics will be available and that we will be in a better position to appreciate the differences which undoubtedly occur in the bacterial content of tropical waters as compared with those in temperate climates and the possible necessity for modifications of existing standards of bacterial impurity.

The significance of *B. coli communis* in water

In the Tropics the water-supply of a town or village is usually derived from one of two sources—from wells, usually shallow wells, and from rivers or streams.

In Khartoum, for I intend to take the problem of the water-supply there as an illustration of the whole question, the supply used to be obtained from both these sources, the river being the Blue Nile—a mighty stream springing from Lake Tsana in the Abyssinian highlands, and fed for the most part by the torrential rains which fall, at one period of the year, on the mountains and tableland of the ancient Kingdom of Ethiopia.

Before, however, entering more minutely into the condition of affairs which obtained and now obtains at Khartoum let us see if there is any recent work to guide us in our consideration of water-supplies in the Tropics, both from a general and from a bacteriological standpoint. As stated, this is not easy to find, but an excellent article on drinking-water in India, and its connection with the subsoil water, is that by Lieut.-Col. Dawson[8] of the Indian Medical Service. He advocates the preparation of contour maps of the subsoil water and deals with the filtering capacity of soil. One point he specially brings out—and it is one with which I must deal later and which

I think has received but scanty notice—and that is, that if the subsoil contains much clay, especially black clay, in which there is a large percentage of organic matter, a process of purification by natural filtration is impossible and there exists, indeed, a culture medium. "Caution must be exercised," he says, "when reliance is placed upon the purifying property of the soil, especially if its characteristics be unknown, for the soil is after all a filter, and all filters, whether artificial or natural, are treacherous, and are a source of danger if not constantly attended to by some competent person." He also comments upon the presence of disused wells in India and their use by natives for insanitary purposes whereby the subsoil water is apt to be contaminated.

The Bacterial flora of tropical water-supplies

Turning now to papers* dealing more exclusively with the bacteriological aspect of the question we find Daniels[11] and Finlayson reporting upon the natural waters of the Federated Malay States. These are jungle streams, and Daniels remarks that the organisms present in such water are abundant, and differ in type from those met with in temperate climates or in tropical climates where the rainfall is less uniform and vegetation not so rank. It is evident, therefore, that no general rule can be applied to water-supplies in tropical countries as a whole. Each must be judged on its merits, and it will be a long time before sufficient facts will have accumulated to enable us to compare the waters of hot, dry, desert countries like the Northern Sudan with those of steamy, humid regions where vegetation is rife and the rainfall is heavy.

As regards samples of water taken from such jungle streams, Daniels considered it advisable to consider as a basis:—

1. The number of organisms exclusive of known, easily recognised, non-pathogenic organisms, *e.g. B. subtilis*, *B. megaterium*, *B. prodigiosus*, *B. violaceus*, and some of the organisms which form characteristic yellow colonies which have been proved to be non-pathogenic to lower animals.

2. The amount of the water required to react, forming acid and gas, with MacConkey's medium, from ·2 c.c. to 2 c.c. being employed in the tests.

3. Indol formation in 48 hours in peptone water with 10 c.c., 5 c.c. and 1 c.c. of the water to be tested.

Bacterial standard of Daniels and Finlayson

As a standard the two observers suggested that:—

1. Not more than 100 organisms, exclusive of those mentioned, should be present in 1 c.c. of the water.

2. That no acid and gas should be formed in 24 hours in MacConkey's medium at 37° C. with 2 c.c. of the water.

3. That no indol should be formed in peptone water in 48 hours with 5 c.c. of the water added.

They remark that the standard is not a high one but that it is exceptional to find a natural water that will pass all three tests. In unprotected shallow wells, liable to be polluted by surface washings, they found the average bacterial count to vary between 300 and 900 per 1 c.c., while in 23 out of 27 wells examined, acid- and gas-forming organisms were present in 2 c.c. and usually in 4 c.c. of the water, and indol formers in 5 c.c. in the case of ten of the wells. *B. pyocyaneus* was found in one instance, and in two cases, organisms indistinguishable from *B. coli communis*.

* The work of Edwards[9] on the water derived from arms of the deltas of Philippine rivers, scarcely merits consideration. He merely says that waters should be condemned (*a*) for the presence of animal parasites (*b*) for the presence of pathogenic bacteria, and (*c*) when the colony count is above 200 per c.c. A paper by Dr. Katrak,[10] in the *Transactions of the Bombay Medical Congress* (1909), is more speculative than helpful.

However useful then this work was for local purposes, it does not help us much in considering the question as a whole, and until recently there were, so far as I know, no statistics of value in this direction. Happily, the first step has now been taken by Major Clemesha[12] and his assistants, who have inaugurated in India a series of observations which may yet prove to be epoch-making and are already highly suggestive.

Clemesha's observations

Let us consider what Clemesha set himself to do and at what conclusions he has arrived. He stated, in the first instance, that there must be considerable doubt as to whether the standards of purity in common use amongst sanitarians in England were suitable to India. The simpler tests, when applied to Indian water samples, showed that most of the latter are loaded with fæcal contamination and that no analyst in England would dream of passing them as fit for human consumption. And yet it is remarkable that the evil results of using such sewage as drinking-water are not always apparent, even on enquiry. True there are outbursts of epidemic cholera; dysentery, like the poor, is always present to some extent, and there is a terrible infantile mortality, but all things considered the results are not in proportion to the cause *at all times*. Again, a great deal of the pollution of surface waters in the East is caused by the excrement of animals, chiefly cattle and goats, whereas in England the pollution of rivers at least has its origin largely in the sewage from towns upon their banks. After these preliminary remarks, and others which need not be quoted, Clemesha proceeds to give an account of the bacteriological methods he and his assistants followed in their efforts to arrive at a suitable standard of purity for the drinking-water in the Madras Presidency.

With these we are not concerned beyond noting that MacConkey's procedure, somewhat modified and including the use of special sugars, was found of great value, that stress was laid on the value of determining the total colonies on agar, that the sporogenes milk test was adopted as was the reaction of Voges and Proskauer, while, as regards indol, the benzaldehyde reaction was employed. Further, the short cuts which have been devised by Houston in his work for the Metropolitan Water Board were found to be of the greatest service. Having had the privilege of seeing Houston's methods in operation I can well believe this to be the case, especially in a hot country where every labour-saving device is to be commended.

The "coli" standard as applied to India

An important chapter in Clemesha's report is that in which he considers the "*coli*" standard as applied to India. He points out how bacteriologists differ in their definition of *B. coli communis*, and he gives the interpretations put upon it by four authorities, namely the English Committee, the American Committee, Houston and Savage. There are two main schools of thought he says:—

"1. The position taken up by Houston, Savage, and nearly all other bacteriologists in England, who apply the term '*coli*' in an elastic sense to an organism defined as above, but who recognise a large number of ill-defined varieties, termed by them '*atypical coli*,' so that the term is in reality made use of to describe a group of varieties, if not different species.

"2. That taken up by MacConkey, Drs. Bergey and Doehan, of the Pennsylvania University, Dr. Orr (in his recent work on the contamination of milk) and ourselves, who apply the name *Bacillus coli communis* to Escherich's organism only, calling all bacilli that differ from it in any 'permanent' test, different species, to which different names or numbers are applied."

"We in India," he continues, "consider the position maintained by the former authorities is open to two objections:—(1) That it is inadvisable to apply the term '*coli*

communis' to a group of organisms, or even so-called varieties, and (2) The tests laid down as essential according to the definitions quoted, do not go far enough; that the list contains too many that are of doubtful significance and utility, and too few that are admittedly of greater importance in the identification of a bacillus. To discuss this last objection first: Houston, Savage, and all experienced bacteriologists admit that the tests given above are not of equal value in the identification of what they term '*coli*.' Thus, to quote from Savage, 'Different characters have not an equal value. Some, such as the fermentation of sugars, liquefaction of gelatine, and acid production in milk are of so permanent a type that for an isolated organism to show divergence from a typical *Bacillus coli* in any one of these particulars would be to throw grave doubt on its being excretal at all, while others, such as the gelatine surface colonies, and the exhibition of motility, are so subject to variation that comparatively little significance can be attached to their, perhaps temporary, absence or modification.' And again in another place he says: 'Organisms that differ from the typical excretal type (*bacillus coli communis*) only in the fact that they show no motility and that their gelatine colonies are atypical, may be regarded as not having their significance diminished on these grounds. Similarly, the loss of indol-production power would not reduce their significance very markedly. On the other hand the absence of glucose or lactose fermentation would at once, in the writer's opinion, exclude the organism isolated from being *bacillus coli* at all.'"

Comparative value of tests

The obvious inference from these opinions is, why not do away with some of these doubtful tests, and substitute for them the more important fermentation reactions in sugars such as dulcite, adonite and inulin? These are entirely on a parallel with the glucose and lactose fermentation referred to as being "of a permanent character." This is practically the position taken up by MacConkey in his paper on the Bacteriology of Milk (*Journal of Hygiene*, Vol. IV., No. 3, July, 1906), and, following his recommendations, by ourselves in the work under report.

He proceeds to illustrate his argument by the results obtained by the application of the proposed methods to Indian waters. It is found that Houston's true *B. coli* can be split up into ten different bacilli, while if his non-typical coli is also considered, more are added to the list, a list which includes *B. grunthal, B. oxytocus perniciosus, B. coscoroba* and others, distinguished so far only by numbers.* According to Clemesha the definitions of Houston and Savage err in omission as well as in commission. He says: "Take the organism *bacillus cloacæ* as an example: it will be observed that this organism does not come within Houston's definition of 'true *coli*,' because it ferments saccharose, nor within Savage's group that indicates fæcal contamination, because it liquefies gelatine, yet it is undoubtedly fæcal in origin. Why should the mere fact that this organism liquefies gelatine mean that it does not indicate fæcal contamination to an equal extent as any non-liquefier? Surely, considering it originally came from the fæces of man or some other animal, the coincidence that it liquefies gelatine cannot put it beyond the range of indicators of fæcal contamination. As a matter of fact *B. cloacæ* is not an indicator of *dangerous and recent* pollution, because it is a resistant organism with a fondness for the bottom of lakes, and river sand; but the liquefaction of gelatine has nothing to do with these facts," and continues:

B. cloacæ

"The weak point of the above method lies in the statement that a large number of what we believe to be entirely different species of bacilli *equally represent dangerous fæcal contamination.* If this be so, it should be demonstrable that these different species are

* For list and tables, see Captain Archibald's paper, p. 319

equally resistant to such forces of nature as the action of sunlight and the action of storage. Otherwise, if it can be proved that one organism is particularly resistant, and another particularly susceptible to these natural forces, it is obvious that the resistant organisms cannot be considered, on all occasions, of equal value, as an index of recent and dangerous pollution, as the susceptible ones. Examining the ten organisms, which fall under Houston's definition of '*coli*,' and which represent his worst type of pollution, we are prepared to state from actual experiments, that the greatest possible variation exists in these ten organisms in their power of resistance to the action of natural forces. To take two extreme examples *B. grunthal* is particularly resistant to the action of sunlight. It is found in waters that have been exposed to the tropical sun for weeks; it is nearly always one of the last bacilli to disappear from a well-sunned and stored water, and also from a mixture of cow-dung and water exposed to the sun. On the other hand, true *B. coli communis* of Escherich (the organism that ferments dulcit and does not ferment saccharose, adonit, inulin, etc.), will disappear with great rapidity from a water exposed to the action of the tropical sun. It is seldom found to survive longer than three days in a clear water of considerable depth. Is it reasonable to include both these bacilli under the same term of 'true *coli*,' and to state that both equally represent objectionable pollution? It is possible to elaborate this still further. In the course of our work we have found that under very exceptional circumstances, a bacillus known as *Oxytocus perniciosus* is occasionally found in a water-supply. There is good reason to believe that this organism is extremely susceptible to natural conditions, such as sunlight, etc., for although it is present in cow-dung, and probably in human fæces, it has only been found in water-supplies after heavy floods, and it disappears with extraordinary rapidity. Thus, it is probable that this organism represents a more recent, and therefore a more objectionable contamination, than any other organism met with up to now. Yet it will be observed that this particular bacillus does not fall within Houston's definition of 'true *coli*,' nor within the category of organisms which, according to Savage, are 'equally of excretal origin.'"

Varying powers of resistance to the sunlight amongst coliform organisms

Such being the case, why, asks Clemesha, do the methods employed in England yield, as they undoubtedly do, such satisfactory results? He answers this as follows:—

"Firstly, the majority of the contamination in surface and river waters is due to human excrement in the form of sewage from towns.

Clemesha's conclusions

"Secondly, the guiding principles that assist a water analyst in arriving at a conclusion, are based on the study of organisms present in sewage, that are human in origin. Thus, the reason Houston gives for saying that saccharose fermenters do not represent so objectionable a form of pollution as the non-saccharose fermenters, is, because the non-saccharose fermenters greatly preponderate in sewage. It should be noted that this kind of reasoning would obviously lead us into serious error in India, where much of our pollution is due to animals.

"Thirdly, there is very little doubt that *coli communis* of Escherich (dulcit +, saccharose, adonit, inulin, Voges and Proskauer —) is probably the commonest organism found in human fæces, and in rivers polluted with town sewage. The work of MacConkey supports this view.

"Fourthly, *B. coli communis* of Escherich is a very susceptible organism to the forces of nature that are inimical to bacilli generally, and its presence therefore does actually represent a recent and dangerous contamination. Evidence in support of this statement can be found in Houston's annual reports, where the ratio between his 'true *coli*,' 'confirmatory' and 'presumptive' tests, varies with the time of the year. Thus, in

summer, the actual number of 'true *coli*' is much less than in winter, due, undoubtedly, to the action of the sunlight in killing off these susceptible organisms.

"Consequently, when an analyst in England finding 'true *coli*' in 1 c.c. condemns a water, because it shows recent pollution, he is right in a great majority of the cases, and in the remainder the worst that can be said of his opinion is that he has slightly erred on the side of over-caution, for it must be remembered that the whole of the 10 organisms comprising his 'true *coli*' are originally of fæcal origin. Now in India, things are entirely different. The *coli communis* of Escherich is a very rare organism in the waters of this country, whereas the other organisms that are included in Houston's definition are extraordinarily common."*

Considering these facts, and also that the bacteriological flora of the fæces of man and animals varies at different times of the year, he came to the following conclusions:—

Bacterial standards must differ in temperate and tropical climates

"1. That standards in use in cold climates are useless and worse than useless in tropical countries.

"2. That it is necessary to separate the individual species of bacilli by well-established tests and to study their characteristics and their position in nature.

"3. That it is advisable to classify all lactose-fermenting organisms according to their ability to resist the action of sunlight and on this to base the standard of bacterial purity."

In the first place, the bacteriology of earth known to be heavily polluted with human excrement was studied.

The results are summed up as follows:—

"1. *Bacillus cloacæ, bacillus grunthal,* bacillus No. 75, and to a less extent *bacillus coscoroba* are capable, under favourable conditions, of remaining alive in the soil for a considerable length of time, probably up to 3 or 4 years.

"2. Fæcal organisms of any kind do not appear to exist in large numbers after they have been in the earth for longer than 1-2 years: night-soil buried in the ground, even in large quantities, loses most of its organisms in this period.

"3. *Sporogenes enteritidis* spores are capable of remaining alive in the ground for a period of something between 3 and 4 years.

"4. *Bacillus coli communis* has never been isolated from the trenching ground samples; hence it is probable, though not certain, that this organism does not remain alive in the ground as long as the more resistant organisms like cloacæ, etc. It has been proved in one experiment to exist in the ground for a period of 97 days."

Next the change in the bacteriological flora of water and fæces during monsoon weather was made the subject of an investigation which showed:—

Bacterial flora in water during the monsoon

"1. That the conditions obtaining during a time of heavy monsoon weather when fresh fæces may find their way easily into water, the whole country being submerged, is in some way connected with the appearance of a set of rare micro-organisms in all the water-supplies over a wide area. This fact has not been noticed with previous and subsequent heavy rain.

"2. That these and allied bacteria may suddenly become extremely common in the fæces of man and animals. The cause of this increased prevalence is unknown.

"3. That flood conditions having passed away, the bacilli very rapidly disappear from all waters. Consequently this supports the suggestion that these organisms are especially susceptible to natural forces inimical to bacteria."

* In this connection one may note that recent work by Castellani [13] goes to show that the typical *B. coli* of Escherich is rare in the intestines of persons living in Ceylon.

Following this, a systematic analysis of the water of a shallow well at the King Institute was undertaken. By a shallow well, Major Clemesha means one which does not pass through an impermeable stratum of soil or rock and is thus liable to contamination from surface and soil washings. It was found that:—

"1. In common with most other water-supplies, including rivers, lakes, springs, etc., *bacillus cloacæ* would appear to be the predominating organism in a well after prolonged drought.

"2. Even a small downpour of rain, after a prolonged spell of dry weather, has a very marked influence in increasing the number of fæcal bacilli in wells situated in porous soils. Systematic study of a "shallow well"

"3. That early showers, following on a long period of hot weather, have caused an increase in the number of *coli communis* in the well of the King Institute. Further investigation is necessary before it can be stated that this is true of all wells. It is not by any means certain where these *coli communis* come from, but it is unlikely that they come from the surface of the ground, and probable that they remained alive in the lower layers of the soil.

"4. That after heavy downpours of rain, in consequence of which it is certain that surface contamination has penetrated deep into the layers of soil, a mixture of fæcal organisms is usually found in the water obtained from wells. *Coli communis* appear to be present after early rains, but disappear as the rains become more plentiful.

"5. Plentiful rains improve the quality of sub-soil water after the contaminated surface water has run off the land.

"6. Shallow wells are a most unsatisfactory source of water-supply, especially if they are situated in a highly porous soil, and unless the greatest possible care is exercised in protecting a large space of ground in the immediate vicinity from all chances of pollution."

Thereafter, the bacteriological flora of the fæces of man and other animals were studied and the following conclusions reached:—

"1. That, the flora of the intestinal tracts of men and animals are subject to very considerable changes due to influences which are at present unknown. These influences have been proved to operate over very wide areas.

"2. That, within certain very wide limits, these forces appear to affect man and animals equally, both as regards number and kind of micro-organisms.

"3. That, under well defined conditions, such as heavy rainfall, the water-supplies contain the same organisms as the fæces of man and animals at that particular time; but that this similarity of bacteriological flora is also noticed occasionally when rain is absent, and there is no apparent cause for it. The explanation of this occurrence is at present unknown. Fæcal bacteria of men and animals

"4. That, having regard to the variation in the bacteria in fæces, both in quantity and kind, no constant approximate composition can be arrived at. Even in the large groups, suggested by MacConkey, variation in percentage composition in the same animal is considerable.

"5. No lactose-fermenting organism has been isolated by us that has been proved to be the inhabitant of the intestinal tract of cattle or man only.

"6. That the numerical relation of the organisms constituting MacConkey's groups in the intestines of cattle in India is entirely different from that in England; while in the intestines of man it appears to be very similar in the two countries.

"7. That a study of the organisms present in fæces at different times of the year is necessary for the proper interpretation of the results obtained from water analyses."

In accordance with his scheme of work Major Clemesha then undertook by ingenious and laborious methods to determine the effect of sunlight on fæcal organisms. From Laboratory experiments it appeared:—

1. That the sun has a very powerful action in destroying all fæcal organisms in water, particularly when they are "naked" and not surrounded by mucus derived from the intestine;

2. That all fæcal organisms do not possess the power of resisting the action of sunlight to an equal degree;

Classes of fæcal organisms

3. That it is possible to divide fæcal organisms, with a reasonable degree of accuracy, into the following classes:—

(i) The delicate organisms or those that are very susceptible to the action of sunlight;

(ii) An intermediate class containing a very large number of organisms which occupy an intermediate position between the two extremes; and

(iii) The resistant organisms or those capable of resisting the action of sunlight for a considerable length of time.

While from weekly analyses of water from a neighbouring lake it was found:—

1. That the action of the sun is powerful in destroying the fæcal organisms and that the surface layers of any large volume of water are in consequence purer than the deeper ones.

2. A practical point, following on this statement, is that the outlet from a reservoir should be arranged as near the surface as is possible.

3. That these analyses show that, in the Red Hills lake, *bacillus cloacæ* is by far the commonest fæcal organism isolated from the bottom.

4. That *coli communis* even in large numbers, in a huge volume of water, disappear with great rapidity. In the results obtained from the Red Hills lake the organisms never survived longer than six days.

5. There is some evidence in these results, though as yet it cannot be taken as thoroughly established, that, under certain conditions, the middle layers of a lake of over 12 feet deep contain more water organisms and more fæcal bacilli than the bottom or the surface.

6. That, when taking samples of lakes, ponds, etc., the depth from which the sample is taken should be carefully stated.

Further investigations on these lines apparently proved:—

1. That the *bacillus cloacæ* and *grunthal* will persist in water exposed to the action of sun and storage for many months, and may therefore be looked upon as resistant to these forces.

2. That the rareness of such organisms as *coli communis* in the samples analysed demonstrates the fact that organisms very susceptible to the action of sunlight do exist; *coli communis* being the commonest example of these.

3. That there exists a large class of organisms, which in point of power of resistance to the action of sunlight and storage, come between the very resistant and the susceptible organisms. This class is made up of many species. Further investigation may make it possible to sub-divide this large group.

Susceptibility to sunlight as a basis for classification

4. That storage, an important factor in which, in the Tropics, is the action of sunlight, is capable of rendering a highly contaminated water extremely pure, and of killing a very great percentage of the fæcal organisms present after gross pollution.

As a result of all this work it was evident that delicate organisms such as *B. coli communis* and *oxytocus perniciosus* exist in nature along with resistant forms, of which

B. cloacæ and *grunthal* may be taken as examples, while, between the two groups, come intermediate species such as *B. lactis ærogenes*. These then are the three classes* into which Clemesha divides organisms which are of importance in the bacteriological examination of water and many of which, let it be remembered, are included in Houston's elastic term, true *B. coli*.

Let us see how he applies his classification to practical purposes, *i.e.* to establishing bacteriological standards for drinking-waters in India.

"All we claim to have established," he says, "is, that in any naturally occurring mixture of micro-organisms, a certain proportion (how great a proportion we have at present not much idea), of one or more species will be found to survive the others when the mixture is exposed to prolonged sunlight. But it is with this resistant residuum that the sanitarian in India is most frequently called upon to deal. Hence, the great importance and utility of this classification." He believes, that in a river in the Tropics, the susceptible individuals among organisms will die off with great rapidity and that the bacilli, which the analyst finds, can be compared with those whose powers of resistance have been studied in the experiments quoted. It is, therefore, necessary that the analyst, besides making himself fully acquainted with local conditions, should always ask himself, "What chances has this sample had of being exposed to the action of sunlight?" As regards the waters themselves, Clemesha considers the three groups as follows:—

Bacteriological standards for Indian waters

1. *Those containing Class I.*—The samples which contain *coli communis* and the members of the susceptible group, present very little difficulty in arriving at a conclusion, as regards the undesirable nature of the pollution. In such samples, derived from river or lake, there can be no doubt we are dealing with recent, and therefore objectionable, contamination. At the same time, if fæcal contamination has been recent, susceptible organisms (those in group 1.) *will be mixed with those of groups* 2 *and* 3, *roughly in the proportion in which they occur in fæces*. Hence we have the double reason for suspecting the water; namely, (1) the presence of susceptible organisms, which shows that the contamination must have been recent and (2) the confirmatory evidence of getting the unaltered proportions of fæcal organisms. In actual practice, *coli communis* is so seldom met with, that the latter condition of affairs is often all we have to guide us. In rivers, organisms in Class I. are more commonly met with, because pollution is constantly being added all along the banks, and, when found, certainly indicate pollution of a most serious and dangerous nature.

2. *Those containing Class III.*—It is probably not realised how extremely often, in a tropical country, organisms, belonging to Class III. alone, are found in water samples. Personally, we doubt whether such a condition of affairs is ever recorded in Europe. But a glance at the analyses of the several supplies, during the quarter of July, August and September, is absolutely convincing on this point. It is quite common to obtain samples of water which contain nothing else but *Bacillus grunthal* or *Bacillus cloacæ*, or a mixture of the two. When this occurs, one may be practically sure that we are dealing with a water from a tank or river that is getting low, and also that these organisms represent the residue of a pollution, months old.

3. *Those containing mixtures of two or more classes.*—The chief difficulty that will be met with by the analyst using this method, will be to arrive at an opinion from samples showing a mixture of organisms in Class II., or a mixture of Class II. and Class III. It is in dealing with such samples that it is absolutely necessary to know the particulars concerning rainfall, etc., that have been enumerated in the early part of this chapter.

* *Vide* Captain Archibald's paper, p. 319

Mixtures of organisms are to be expected in rainy weather and flooded rivers. In attempting to arrive at a conclusion one has to be guided by (1) the number of fæcal organisms present in the sample; (2) the number of different species present in the 10 colonies isolated; (3) whether the organisms show a tendency to belong to those at the top or at the bottom of the list given in Class II.; and (4) whether Class III. are present in considerable quantity. Thus, if in a water containing a hundred fæcal organisms per c.c., known to have been taken from a lake or river within two or three days of a heavy downpour of rain, we were to find four or five different varieties of organisms all belonging to group 2, we should be justified in considering that, although no organisms belonging to Class I. happened to be isolated, the circumstances were highly suggestive of recent fæcal contamination. In such a sample as this, one would be guided by the relative number of such organisms as *Lactis ærogenes*. It has been shown that, in the ordinary fresh mixtures of fæces and water, *Lactis ærogenes* is not particularly common. Therefore, a water showing a mixture, rich in varieties, and yet containing few or no *Lactis ærogenes*, would be judged to have undergone very little settlement or purification.

If, on the other hand, one found the sample containing fæcal organisms in 1 c.c., showing only 2 or 3 varieties, one of which belonged to Class III., and if the predominating organisms happened to be *Lactis ærogenes*, this would constitute fairly satisfactory evidence that a considerable amount of purification had gone on, since the fæcal matter was actually added to the water. The work given in Chapters IX. and X. shows conclusively that, in all waters stored for some time, *Lactis ærogenes* is a common organism, and that it is not so, in newly contaminated waters, nor in waters stored for many months.

Numerical standards

This may be taken as the rough idea as to how conclusions can be arrived at in the types of results commonly met with. On the subject of numerical standards, no hard and fast rules can be laid down, and a considerable amount of latitude must always be left to the discretion of the analyst. In the above remarks it is presumed that, besides the actual organisms found in each sample, the number of lactose-fermenting organisms per c.c., and the total colonies in 1 c.c., are taken into consideration. The results obtained in the bile-salt broth tubes are also very important. Thus, practically any water in India that contains no fæcal bacilli in 20 c.c. is probably a good water (provided, of course, none of Class I. are obtained in a large amount).

In summing up, he says of river waters :—

"*Good river water* should not contain more than 100 colonies (on agar at 37° C.).

"Fæcal organisms should not exceed 1 in 10 c.c.

"No organisms of Class I. should be present in 50 c.c.

"Fæcal organisms present should belong to either Class III. or the more resistant group of Class II.

"*Fair or usable river water* should not contain more than 300 colonies (on agar at 37° C.).

"Should not contain more fæcal organisms than 1 in one c.c., and no organisms of Class I. in less than 20 c.c.

"The fæcal organisms present should consist mainly of mixtures of Class III. and Class II., and there should be a tendency for one organism to preponderate.

"*River waters should be condemned if* total colonies are more than 800 (on agar at 37° C.).

"If lactose fermenters are present in number of 10–100 per c.c.

"If organisms belonging to Class I. exceed 1 in 5 c.c.

"Or if the fæcal organisms isolated (Class I. being absent) are rich in varieties such as occur in an emulsion of fæces."

And then, turning to well and spring waters, he remarks :—

" *A good water* should contain no *fæcal bacilli* in 20 c.c.

" No Class I. in 100 c.c. Total colonies under 50 per c.c.

" We are for the present unable to give any other standards than this."

Now it may be thought I have dealt at too great length with Clemesha's work, but it was essential to do so as one is anxious, if possible, to obtain expert opinion on it.* It has been no easy task to keep the résumé even within these limits, but I trust that all salient points have been considered and his arguments made clear. We have now reached the point at which I was aiming, namely the fact that Clemesha confesses that he cannot tell how far his method is applicable to the waters of springs and wells which are not exposed to the direct action of sunlight. He admits that in these cases the question is much more complicated than when one is dealing only with surface waters, the difficulties being (i) to estimate the importance and significance of organisms that remain alive in the soil for long periods and (ii) to be quite certain when a pollution is caused by surface contamination washed down through cracks or through a porous soil.

Importance of quantity in pollution

The quantity of fæcal pollution, he regards as of paramount importance in the case of well-water, and the standard he adopts has been given. This of course applies only to surface wells. He tells us nothing regarding deep wells, and here it is that I hope to make good a deficiency and to show that in Khartoum, at least, the standard of Houston and Savage has proved itself a reliable guide in an interesting and difficult problem. I hold no brief for Major Clemesha and his work. It requires confirmation and may be refuted. Captain Archibald has been working† on similar lines with river and shallow well-water in Khartoum where, however, conditions differ considerably from those in Madras. But whatever may eventually be proved, there can be no doubt that the Indian work is in the right direction and that it has been carried out with much care and devotion and in an eminently sensible and scientific manner.

To revert to Khartoum. At the time when it became necessary to embark upon water work there, Clemesha's researches had not appeared, and one had to proceed more or less on the old familiar lines.

The Blue Nile water

I have mentioned the Blue Nile, a river clear when low, with very palatable water of an agreeable softness and colour, and comparatively pure save where fouled from the bank at a town or village. I need not enter here into the chemical composition of its water.‡ In flood time it is, of course, muddy, laden with silt and washings from the Abyssinian mountains. Some records of bacteriological observations are available though several were destroyed in a fire. Those existing show that in January, 1907, at low Nile, the number of organisms in 1 c.c. of the water taken close to the bank above the town was between 300 and 400 (agar count at 37° C., 48 hours' incubation), while in a sample taken from the centre of the stream at Buri in February, 77 organisms were found per 1 c.c. In May, with the river rising, a sample taken from mid-stream, opposite the Gordon College, gave 84 organisms. Other observations show that the counts vary considerably and close to the bank as many as 700 colonies per 1 c.c. have been found, while *B. coli* of an excretal and "flaginac" type is commonly present in from ·5 to 1 c.c. of water taken close to the bank, but is not found in less than 5 c.c. of water from mid-stream. Formerly, the Nile served as the chief source of supply so far as drinking-water was concerned, and so long as the water was not stagnant did not

* This is now to some extent forthcoming in a recent paper by Brevet-Colonel R. H. Firth *(Journal Royal Army Medical Corps*, November, 1910*)*.

† *Vide page* 319.

‡ *Vide* paper by Dr. Beam in Second Report of these laboratories, p. 207 *et seq.* Also, and in fuller detail, in the Third Report, p. 386.

appear to be a cause of communicable disease, for reasons I have stated elsewhere (*Third Report, Wellcome Tropical Research Laboratories*, 1908, p. 84).

Water for washing, cooking, irrigating gardens and watering animals was, in the main, derived from the numerous shallow wells with which the site of the town is honeycombed. Such water is always impure from a chemical standpoint and also from what we may call the English bacteriological standpoint. It is hard, and often contains *B. coli* of a "flaginac" and excretal type in so small a quantity as 0·02 c.c. I have known *B. pyocyaneus* to be present in such water and have also seen its use result in dysentery, and systematic pinking of such infected water led to a cessation of the cases it had caused. In this connection I show a drawing (Fig. 95) of an excellent type of apparatus for taking water samples from wells and rivers, of a type not figured in the text books.

Collection of samples

It will be seen that it consists of a metal stirrup on which the collecting bottle, of 250 c.c. capacity, rests. A steel rod is attached to the stirrup, and, by screwing lengths of similar rod together, any depth, up to 50 feet or so, can easily be reached. A ring support holds the bottle in place and through other rings a wire runs. This wire is attached to a pair of flexible metal forceps which grasp the flat bottle stopper, and are operated by a circular collar which can be slid up and down. The stopper has a glass rod attached to it and when the former is drawn out by the attachment, and checked from coming up too far, by one of the metal rings, the glass rod is not wholly withdrawn from the bottle neck and so guides the stopper back into its place.

The whole apparatus can be sterilised by the flame of a blast lamp and is light, easily handled and exceedingly convenient.

It is a copy of one I saw in use in Vienna and was made for me by Messrs. Baird & Tatlock, Ltd., of London.

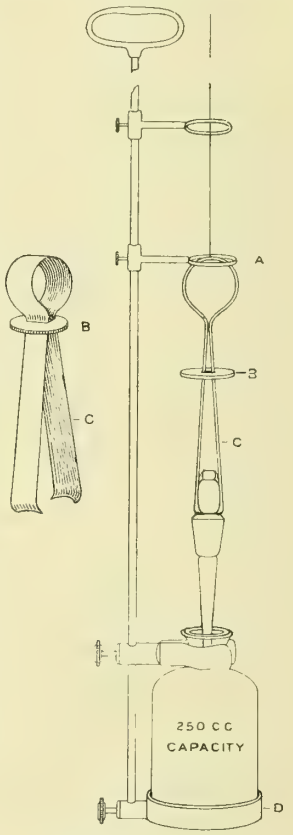


Fig. 95.—Apparatus for collecting water, at various depths, from wells and rivers, for bacteriological analysis

A—Check ring
B—Sliding collar
C—Flexible metal grip
D—Stirrup for base of bottle

When the question of an improved water-supply came up for consideration, it was evident that only two sources were available.

1. The river; the supply to be taken above the town.
2. Deep wells, if such could be obtained.

The advantages of the river supply were, that the water was known to be palatable, that there was an unlimited quantity, that it would be popular with the natives and that the works required were not likely to be expensive. Its disadvantages were the necessity for the construction of settling tanks unless the water could be obtained through pipes sunk in the sand-banks, or by means of galleries beneath the river bed. Moreover, as the river water was certainly liable to pollution from the banks and from boats and steamers it could not be relied upon as a permanent supply without filtration, and filters, even mechanical filters, are to be looked upon with suspicion especially in a country where native labour has to be employed.*

* This was prior to the employment, on a large scale, of ozone or the action of the ultra-violet rays for water sterilisation.

The advantages of a deep well supply were, that in all probability a clear and pure water would be forthcoming and that such water would be safe from pollution and would not require filtration. Its disadvantages consisted in possible heavy expense and in the chance that the supply might be inadequate or the quality unsuitable. It might be excessively hard or contain iron in undesirable quantities, or be of such a nature as to require special treatment. In short, though one knew where one was with the river supply, one was totally in the dark as regards the deep wells, for nothing was definitely known regarding the geological formation, and there was nothing to guide one in a new country like the Sudan.

After some discussion, it was decided to have a trial boring sunk, which would both furnish information regarding the strata and enable samples of water to be obtained for chemical and bacteriological examination. At the same time I set on foot some enquiries into the flow of the subsoil water and its relation to the Blue Nile. A site was selected above the town and close to the southern bank of the Blue Nile, and operations were commenced with great energy. Indeed, so great was the energy displayed that instead of a single trial bore, the whole of an elaborate scheme of well-sinking was carried through; nor could it be checked despite protests and representations from those responsible for the public health. Proper samples for analysis could not be obtained for months, and when at last the engineers declared themselves ready to submit such samples, it was found that six wells had been sunk, but as a seventh was afterwards drilled it will be convenient to describe the installation at a period just prior to that when the final tests were made.

Khartoum water-supply experiments

The site has already been stated. It suffices to say that no great distance away lies the village of Buri which is served by shallow wells liable to pollution.

The water works, as will be seen (Fig. 94, *page* 288), consisted of seven wells and a pumping plant. Since then, a large, covered reservoir has also been provided. The wells lie along two lines, one at right angles to the river, the other nearly parallel to it. Well No. 5, in the first line, is 30 yards from the river bank. The wells are about 60 metres apart, are drilled, and at that time were of the following depths, *i.e.* Nos. 1, 2, 3, 4, 5 and 6 of 75 metres, and No. 7 of 176 metres, these depths being, to all intents and purposes, from ground level.

Wells Nos. 1 to 6 were alike in being fitted down to a depth of 22·40 metres, with a casing pipe of $9\frac{1}{2}$ inches diameter. Below this point, the bore-hole, 8 inches in diameter, extended, unguarded, to the full depth of 75 metres.

Well No. 7 was cased with a $6\frac{5}{8}$ inches pipe down to 54 metres, while the unguarded bore-hole, of $6\frac{1}{4}$ inches diameter, extended below this to the full depth of 176 metres.

As has been said, there was originally no reliable information regarding the geological structure of the site. Some details had been gathered and sections prepared when Well No. 1 was drilled, but as there was no core-boring and the material was washed out in a finely comminuted state, the information furnished could not be regarded as very reliable. Indeed, subsequent observations proved it to be incorrect in certain particulars.

So far as could be told the upper strata down to a depth of 23 metres consisted of layers of sand, clay, mud and gravel. Immediately below, came rock seamed, so it was said, by beds of clay. This rock, there was reason to believe, was a porous limestone, a "Travertine," but at the time the first tests were made, no information was forthcoming regarding its nature. It was understood, however, that the casing pipes ended in it and not, be it noted, in any impermeable bed of clay. At first, indeed, this rock was said to be sandstone with a layer of clay marl overlying it, and it was assumed that a more or less similar formation existed in the case of each of the first six wells. Anyhow, below this doubtful

Geological conditions

stratum was the water-bearing sandstone which was shown as coarse and containing gravel. The earlier sections showed the rock to be seamed at different levels with clay marl.

There had apparently been a fault between Well No. 7 and the other wells, for, in its case, below 23 metres, the strata differed in position and thickness. One need not, however, go into details, nor is it necessary here to enter into the question of the quantity of water yielded at first by these wells, as our space is so limited, but a word or two may be devoted to the air-lift pump, the method of water-lifting eventually employed. The air-lift, which is not strictly a pumping machine, was, I believe, originally an American invention which, under certain conditions, renders excellent service. Its principle of action is the lessened specific gravity of water in which air is contained in considerable quantity, or through which it is rising in bubbles. There is an air-pump or compressor, and from it an air-pipe passes to the bottom of a water tube, into which tube air is discharged. The air rising, causes the surface of the water to rise, since the atmospheric or other pressure at the base remains the same. The output from deep wells can thus be increased although the total mechanical efficiency of such a plant is said to be less than that of a good pumping installation. One mentions it here as, naturally, its influence on the bacterial content of the water required consideration.

The air-lift pump

As may be surmised, the very scanty information obtainable regarding these wells did not place a bacteriologist in a very favourable position for coming to definite conclusions regarding the results he might obtain on analysis. The responsibility was great, the problem novel, and there was determined opposition to sanitary interference. One could not tell if heavy rains, falling on a cracked and gaping surface soil, might not wash impurities to such a depth that they could enter the well-water by the unguarded bore holes; one could not tell if, under the influence of powerful pumping, with great depression of the water table, river water or water from pools in the dried river bed might not similarly gain entrance. From the river bed to the top of the unprotected bore hole of Well No. 5 there was only 13 feet of sand in a vertical direction, and 90 feet of presumably porous material in a horizontal direction to act as a "natural" filtering medium. Again, there were the shallow native wells in the neighbourhood which certainly merited some consideration. One felt doubtful about the whole scheme. There might be little evidence of pollution and yet the water might, under certain conditions, be a source of danger. One had been confidently assured that the water was the finest in the world and had replied with the expressive Arabic word "Yimkin," which, being interpreted, signifieth "perhaps." If, at first, the results were good, it might be difficult to prevent the water being rushed upon the town although it was evident that the tests would have to be prolonged and careful. The influence, if any, of the falling and rising Nile, upon the wells, certainly called for study, as did the effect of severe and exhaustive pumping.

Doubts as to the quality of the supply

It was, I confess, an anxious time, and one was scarcely prepared for the results which were obtained. Before mentioning these, one must outline the methods of examination employed.

Water was collected from the wells in special sterilised glass-stoppered bottles. The discharge was, as a rule, from a special wrought-iron pipe of small diameter, leading from the large delivery-pipe directly connected with the well. The small pipe was passed through the furnace every morning of the days on which samples were taken. Its faucet was also flamed prior to collection of the samples. As a rule, three samples were taken from each well and at least two of them were examined. The bottles, after their necks and stoppers had been flamed, were filled, packed in ice and transported by motor or launch to the laboratories, where the examination was commenced as soon as possible, nearly always within half-an-hour of the time of taking the samples.

Quantitative Examination

The samples were plated on the usual nutrient agar (1 per cent. to phenol-phthalein) and the quantities usually employed were ·2 c.c., ·3 c.c., ·5 c.c. and 1 c.c. Occasionally, 2 c.c. plates were also poured. The plates were made in duplicate and often in triplicate. As a control, one set of plates was poured by me from one water sample and another set by my assistant from another sample. Now and then, the third sample was also utilised for making plates. In addition, control plates were made with agar alone and with agar to which sterile water was added. The plates were incubated for 48 hours at a uniform temperature of 37° C. and then counted. Occasionally, there was trouble with the incubator, and when a temperature of 37° C. could not be maintained, the plates were allowed to remain for a longer time in the incubator. Technique

As a rule, the technique proved satisfactory, but on one occasion it was found that the stock agar had an inhibitive action on bacterial growth, owing, probably, to faulty preparation or standardisation, and the counts made on this agar were discarded.

Further, on several occasions the presence of haboubs, or high winds with dust, resulted in air colonies getting access to the plates and ruining the work. It was found impossible to prevent this contamination.

Qualitative Examination

The presence or absence of *B. coli* was determined at every examination. For this purpose the following standard media were employed:—

Glucose, bile-salt neutral-red broth with Durham's tube for gas formation, MacConkey's glucose, peptone, bile-salt medium with Durham's tube, and latterly the new lactose-bile medium of Jackson with Durham's tube, which is said to constitute the most delicate presumptive test for the presence of *B. coli*.*

The quantities of water examined were as a rule 5 c.c., 10 c.c., 25 c.c., 50 c.c. and 100 c.c. When any suspicious appearances were observed, confirmatory tests were employed; the organisms present being plated out on the Endo or Drigalski-Conradi media or on lactose bile-salt neutral-red agar, and then examined for indol and subcultured into the usual milk, sugar and litmus media as well as examined microscopically.

I may say that, on the whole, the Endo fuchsin agar yielded the best results. When considered necessary, the *B. enteritidis sporogenes* test was applied and a search made for streptococci. When essential, the pathogenic effect of a suspicious microbe was tested on animals—gerbils, jerboas, fowls and guinea-pigs. In short, the method favoured by Savage was closely followed. Savage's method closely followed

The condensation water in the air-pipe line, serving the air-lifts, was also examined bacteriologically.

Routine observations, as regards the temperature of the water for different wells tested, and as regards the level of the ground water in the wells, were made while the tests were being conducted. The data as regards levels were furnished by Mr. Williams—the Engineer of the Water Works.

I have said that the results of the first examinations were surprising. They were such as to lead to an immediate condemnation of the water both on bacteriological and chemical

* This has recently been confirmed by Hale and Melia,[14] who also state that lactose-bile makes a distinction between recent and distant contamination, and hence gives better evidence of the actual relative sanitary quality of a water. Fromme (*loc. cit.*), however, is not of this opinion.

grounds. Samples were found to be faintly opalescent, becoming more so on standing, and depositing a very small amount of sediment. The taste was faintly chalybeate. The colony count varied between 1000 and 2000 per 1 c.c. *B. coli*, as a rule of a "flaginac" and excretal type, was present in 1 c.c. and larger quantities, the free ammonia figure, ·5 to ·6 parts per million, was excessively high, and iron and manganese were present in objectionable quantities.* 1·33 parts of iron per million and 0·17 of manganese clearly indicated that there would be trouble with crenothrix growth in pipes to which such water was admitted, and this proved to be the case, *C. polyspora* speedily making its appearance.

Danger of crenothrix

According to Hazen, ·5 part of iron per 1,000,000 is about the permissible limit, while, in the Rod-El-Farag well-water in Cairo, trouble had ensued with ·35 part per 1,000,000 of iron and ·77 part manganese.

Dr. Beam, our Chemist, and I, reported that, as a result of the examination and a study of the topography of the well district, we were led to believe—

1. That the iron and manganese are derived from strata and will persist in the water.

2. That we are dealing with a mixture of deep well-water whose source is the Blue Nile and of superficial or ground water highly charged with micro-organisms which is gaining access to the wells either through defective joints in the pipes or, what is more likely, through cracks and fissures in the strata or through porous beds.

Naturally this adverse report did not please those who had forced on the water scheme without due precaution, deliberation and the necessary enquiries and tests. Only one well had been tested, and the conditions of sampling were said to be unsatisfactory.

Pumping trials and the results

Proper pumping trials with ordinary pumping apparatus were now commenced, the mixed water from 5 wells was examined with every precaution, and the results, as will be seen from the conclusions to our report, were much the same. We stated that—

"1. From a physical standpoint it is apparent that the water is quite unsuitable for a town supply. It is not clear when freshly pumped, and becomes distinctly turbid on standing. Moreover, as predicted, the presence of iron and manganese in the water has resulted in the rapid growth of the crenothrix fungus and the development of an odour distinctly disagreeable. The taste is also somewhat unpleasant.

"Such a water would infallibly cause serious trouble by leading to a blocking-up of the service pipes, especially those of small calibre. The greatest trouble would result when the iron-stained filaments of the fungus break off and become diffused throughout the water. This renders the water quite unfit for laundry use owing to the unsightly and destructive deposit of iron mould on linen and other fabrics. Moreover, it is not to be expected that people would pay water rates for water of this character.

"In this connection we may cite the so-called 'water calamity' of Berlin, and the similar calamities recorded from Rotterdam, Charlottenburg, and many other places, including, to come nearer home, the recent revelations in Cairo. It is true that aeration combined with filtration will, in most cases, remedy this evil, but the filtration must be efficient, as there is already a case on record of an incompletely filtered water, from which the iron was not wholly removed, resulting in the growth of crenothrix.

"2. From a chemical standpoint the water still remains much harder than that of the Blue Nile, and a soft water is always preferable for a town supply, from an economical standpoint, owing to the action of hard water on boilers and soap. The presence of iron is, of course, objectionable chiefly for the reasons already detailed.

"In our former report we mentioned that the quantity of iron is far in excess of what is permissible in a town supply. The slight reduction in total solids and carbonates indicates that the forced pumping has resulted in the drawing in of a larger amount of the water to which the contamination of the deep well-water is due.

"3. From a bacteriological standpoint the water is in much the same condition as it was at the time of the former examinations, so far as the presence of *B. coli*, the standard bacterial indicator of impurity, goes.

"The number of organisms present in 1 c.c. is greater than that present in the last sample taken from Well No. 3, on Jan. 19, 1908. The results of the examination of this sample have not been submitted to the Board, as the analysis was still proceeding at the time of the last meeting. 218 micro-organisms were then found per 1 c.c. and true 'flaginac' *B. coli* was present in 1 c.c. of the water.

"Hence, there has been very little change, and what change there is has occurred in the wrong direction. In a pure, deep well-water the bacterial count at blood heat should not exceed 5 to 10 micro-organisms per 1 c.c.

"As stated, *B. coli* is taken as the standard of pollution, and the presence of *B. enteritidis sporogenes* merely serves to add confirmation to the evidence of pollution.

* Much free ammonia unaccompanied by "albuminoid," however, as is well known, is frequently found in waters containing iron and is derived from the reduction of nitrates.

"This organism should not be present in less than 1000 c.c. of deep well-water. Here it has been found in 500 c.c. In shallow well-waters it should not occur in 100 c.c., hence it was not necessary to test for it in lesser quantities than half a litre.

"As regards *streptococci* they have much the same significance as *B. coli*; in other words, when present in large quantity they indicate fæcal contamination, but their position as precise indicators is not yet assured. It will be noted that they were present in 0·1 c.c. of the water. As compared with Blue Nile water it is evident that, bacteriologically, the samples of well-water examined were just about four times as bad."

I was now somewhat in the position of Pooh Bah in the "Mikado." As Government Bacteriologist I condemned the water and as Medical Officer of Health I refused to accept it. Personally, I was inclined to make a stand for river water and filtration, or at least to insist on filtration of the well-water.

With the scanty information available, it was impossible to come to any definite conclusion regarding the source of pollution. Noting, however, a certain ratio between the numbers of micro-organisms present in the well-water and those in the Blue Nile water I concluded that the river water was finding its way into the wells and, considering Dawson's views, which I have already cited, I hazarded the opinion that one or other of the soil layers was acting as a culture medium and that, under conditions of increased temperature and favourable nidus, the organisms in the river water were multiplying *in situ*. The type of organism found on the plates favoured this hypothesis. That this view was in all probability correct I may at once declare, for further investigations proved the existence of a bed of bluish shale.

Probability of bacterial growth in soil layers

As there was also a doubt regarding the quantity of water available, it was decided to obtain the advice of Mr. Abel, of Cairo, a well-known hydraulic engineer. He was accompanied by Dr. Todd, of the Egyptian Public Health Department, who acted as Mr. Abel's personal adviser on the bacteriological aspect of the question. Their visit unfortunately took place at a time when the laboratories were partly gutted by a disastrous fire, and Dr. Todd's work had to be limited, but they had the advantage of being given every facility and full information, as it was hoped their investigations would put a different complexion on the case, especially as the air-lift plant had been installed and was in use. So far as the chemical and bacteriological analysis went this was not the case. The work done in the laboratories was amply confirmed, and their report only differed from ours in declaring that any true surface contamination was impossible. In this, I believe, they were perfectly correct. Further acquaintance with soil conditions has assured me on this point, though my chief fear was that water from polluted pools in the river bed at low Nile might find its way into the unprotected bore holes.

Report of Mr. Abel and Dr. Todd

One cannot enter into their interesting enquiries and elaborate tests in detail, but it is useful to tabulate their results. These are as follows:—

"The results of the bacteriological tests show that at the time of the trials the water was far from satisfactory from a hygienic point of view.

"The two wells, which were subjected to a more or less detailed examination, gave evidence of a somewhat intense and irregular pollution.

"For reasons given above, it was not possible to definitely locate the source of the pollution; but, from the results of certain tests, we were forced to the conclusion that the water which enters the wells from some of the upper layers tapped, must be highly contaminated.

"How this contamination arises is a question which we are unable to settle, from want of sufficient data; but we are convinced that any true surface contamination in the immediate vicinity of the wells is out of the question.

"Concerning the chemical constitution of the water, we are not inclined to lay much stress on the results of the analysis available, as these are hardly sufficiently numerous, nor do they cover a long enough period.

"They, however, appear to show that, apart from the presence of iron and manganese, no serious objection can be raised from a purely chemical point of view.

"Should iron and manganese ultimately prove to be present in quantities large enough to create difficulties, these would in no way be insurmountable, and the question would be merely one of expense."

Their recommendations are of special interest. They advised:—

"1. The cutting off of the upper strata in the wells down to a certain depth.

"2. The improval of the compressed-air plant, so as to guarantee continuous working and avoid contamination of the wells from the air-lift.

"3. A continuous pumping trial, extending over a period of at least a fortnight, with regular measurements of the output and depression.

"4. A series of bacteriological and chemical tests made at suitable intervals throughout the above trials.

"If these tests give satisfactory results, so that regular pumping from the wells is begun, the following might be proceeded to:—

"5. A periodical bacteriological control.

"6. A regular chemical examination of the well-water with a parallel examination of the Nile.

"7. The deepening of Wells Nos. 5 and 6 to about 200 metres.

"8. The boring of a new well at a point suggested and taking advantage of this boring in order to obtain accurate information both as regards the various strata and as regards the water carried by these."

I may say that they were led to believe that the sandstone layers belong to the Nubian sandstone—which is said to reach from Uganda to Assouan, and that they are therefore extensive water-bearing strata fed mainly by seepage water from the Nile. Further chemical examinations have led Dr. Beam and myself to regard this opinion as correct. The deep water is probably derived from the White and not the Blue Nile, and it is likely that it has travelled a long way underground.

Messrs. Abel and Todd, who were put in possession of information regarding the "Travertine" bed of which we were ignorant, made the interesting discovery that strata of similar material crop out on the banks of the river east and south-east of the town—it is therefore possible that this layer places the wells in direct communication with the river, the porous bed extending some two miles or so from the site of the wells to the river outcrops. This and the presence of the bed of bluish shale, which was found to exist immediately beneath the "Travertine" and to be about 6 metres in depth would, in my opinion, adequately explain the results at first obtained, *i.e.* the evidence of a mixture of pure deep well-water and of water which, from the English bacteriological standpoint at least, was grossly polluted. Granting these conditions exist, could such water travelling into the wells from the river cause disease under any known conditions? I believe it might. There are native villages above the outcrops, and there is a certain amount of boat and steamer traffic. Given enteric fever, dysentery or cholera, I do not see why specifically contaminated river water might not reach the wells. It is true that in the bed of bluish shale saprophytic organisms might successfully combat the pathogenic, but we know that *B. coli communis*, at least in some of its forms, is not a very resistant organism, and yet it was present in very small quantities of the water. In the case of Nile water also one must not forget such a disease as dracontiasis, while the recent confirmation of the correctness of the Mills-Reincke phenomenon is another strong argument in favour of obtaining as pure a water-supply as possible. At any rate, as will

Geological conditions point to the possibility of pollution

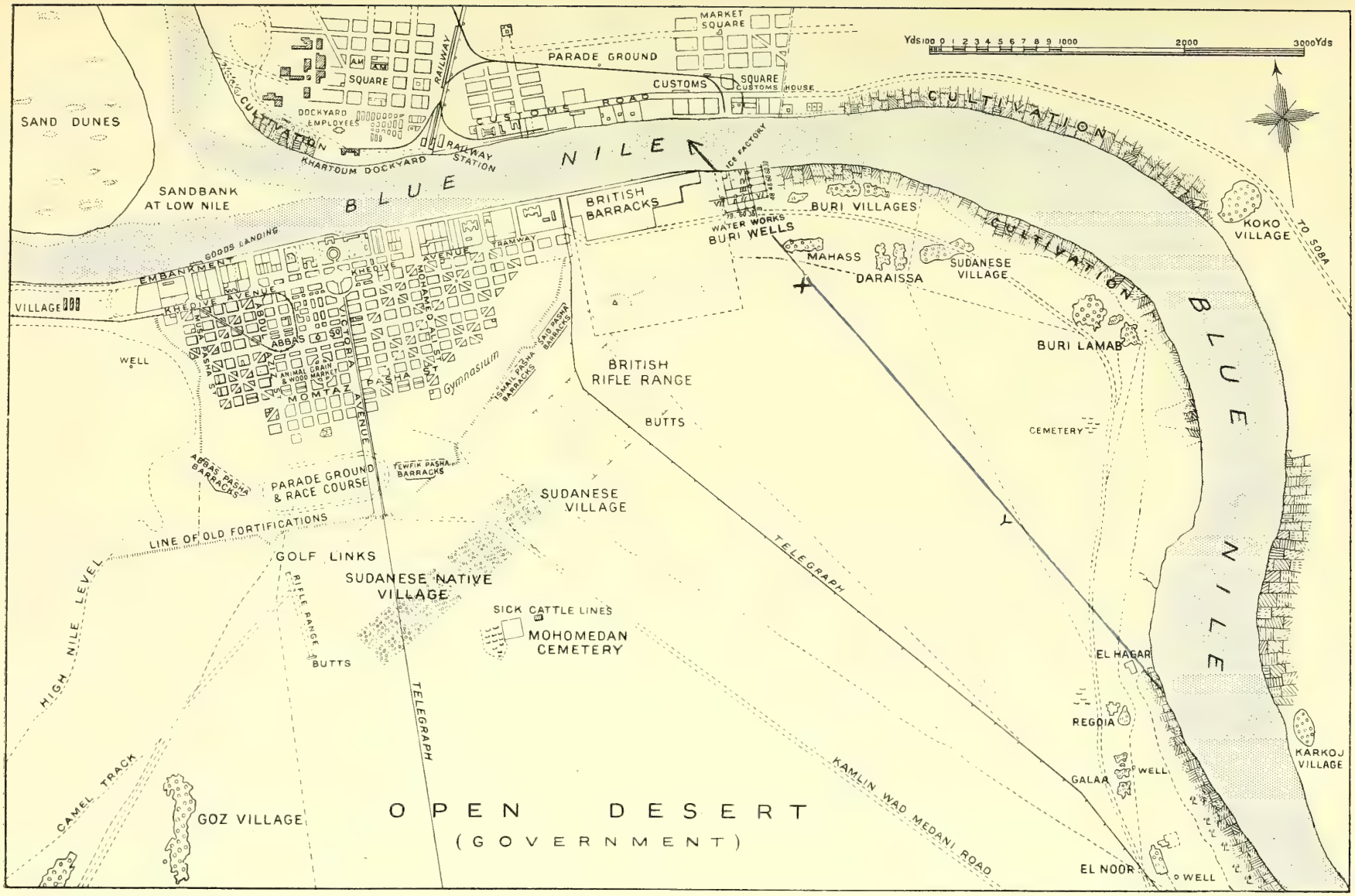


FIG. 96.—MAP OF KHARTOUM SHOWING PROBABLE LINE OF PERCOLATION (X—Y) FROM BLUE NILE WATER ALONG STRATA OF "TRAVERTINE"
This was determined by observations on the flow of water in the "Travertine" bed
From plan prepared by Mr Williams and reproduced with the kind permission of the Director of Works

be shown, *B. coli* gave clear indication of the contamination or, if you will, admixture of the true deep water with superficial water. Even supposing the *B. coli* found were to fall wholly into Clemesha's third group, I think it would have been the height of absurdity to pass wells which at very heavy expense presented the town with water—one half of which at least, let us say, was derived from the very river which flowed past 30 yards away from one of them. This, too, apart from the important question of the contained iron and manganese.

Happily, the laboratory representations prevented this water being supplied to the town, and in my Public Health Report for 1908, I wrote

"one can only hope that some means will be devised whereby the water may be obtained free from chemical and organismal impurities.

"If this is found impossible then filtration must be employed or, if necessary, the Blue Nile water utilised and passed through bacteriological filters. The threatened invasion of cholera last year renders this water-supply question most urgent and important."

The difficulty solved by cutting off upper strata with cement

Fortunately, the question was solved, and satisfactorily solved, along the lines suggested by Messrs. Abel and Todd. The upper strata were cut off with cement, and as a result the colony count fell nearly to zero, *B. coli* vanished, and the quantity of iron and manganese greatly diminished. As this engineering device may not be generally known to medical men it may be well to indicate how it was carried out. I am indebted, for particulars, to Mr. J. E. Williams, the engineer in charge of the works who, at all times, was most courteous and obliging. The process is simple enough. As noted, with the exception of No. 7, which was of smaller bore, the wells down to a certain depth were lined with a casing tube of $9\frac{1}{2}$ inches diameter. A second tube of $6\frac{5}{8}$ inches diameter was passed down each well to the depth where it was desired to cut off the upper strata, *i.e.* to one or other of the deep beds of impermeable clay. This inner tube, therefore, descended in the open bore-hole below the level of the end of the outer tube. In order to facilitate operations, the well was first of all filled with sand which provided the necessary cushion. Liquid cement was then poured between the two tubes down to the depth desired, and, in cases where it did not settle properly, it was blown down and packed by means of compressed air. It set for the most part under water and formed a hollow column or ring, the lower portion of which, confined between the inner tube and the wall of the bore-hole, in some degree soaked into the porous sandstone with which, as stated, it was in contact, and rendered the latter absolutely impermeable. Although transverse fissures may occur in such a column, it is scarcely conceivable that any lengthy vertical crack can be produced in it. Hence the cutting off is regarded as efficient. Finally, the cushion of sand was blown out of the well which, if the clay bed is both extensive and impermeable, is thus wholly protected from any local influx of water from upper and possibly contaminated strata.

Conclusions

I will now conclude what, despite many omissions, has proved a somewhat lengthy paper, by quoting from portions of my final report to the Sudan Government on the examination of the well-waters before and during special and continuous trial pumpings of twenty-one days duration in the months of March and April, 1909.

"The wells tested were Nos. 1, 5, 6 and 7, and the first three at the time of the special pumping trials had undergone very extensive alterations since the examinations conducted in 1907-8. For one thing the air-lift pumps and air compressors had been introduced, while Well No. 5 had been deepened, and, in all, the upper strata had been cut off by the special process. No. 7 was a new well to us. It had been bored since the last examination. As a result of these changes we soon found that we were dealing with quite a different water from that which had led to a condemnation of the original wells. Nos. 2, 3 and 4 have been discarded and filled up with sand.

"As, from previous experience, confirmed by the results obtained by Messrs. Abel and Todd, we were aware that the old wells had been contaminated by the influx of polluted water from the upper strata, it was of the highest importance to ascertain.

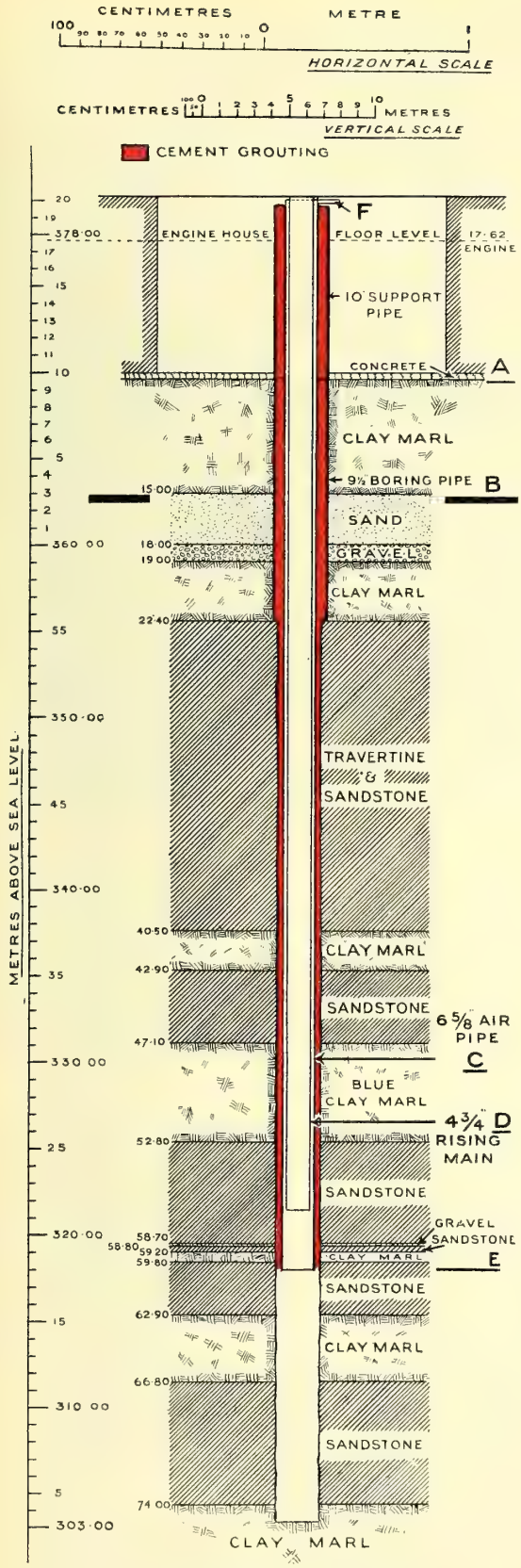
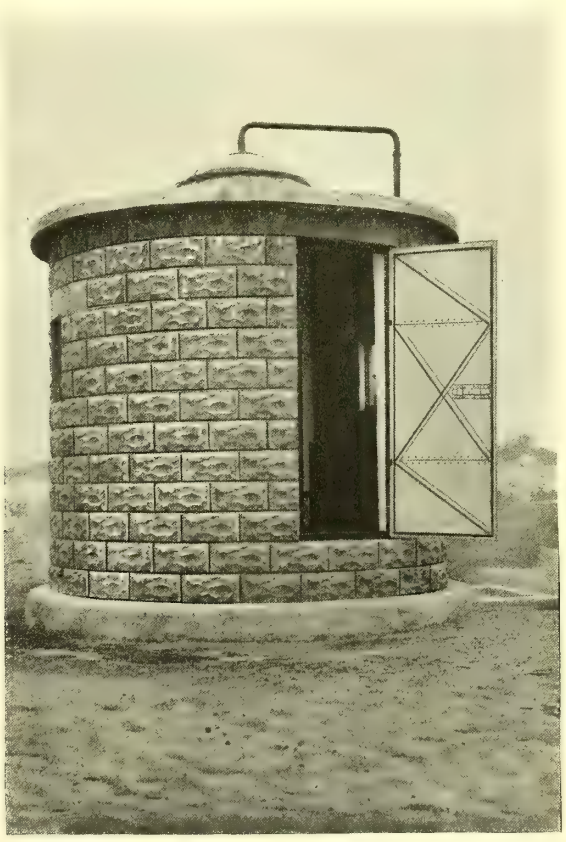


FIG. 97.—SECTION OF WELL NO. 6
BURI WATER WORKS, KHARTOUM
The cement grouting entirely prevented contamination from Blue Nile Water along strata of "Travertine"
From plan prepared by Mr. Williams and reproduced with the kind permission of the Director of Works



G. BUCHANAN

FIG. 98.—WATER TOWER



G. BUCHANAN

FIG. 99.—WELL HEAD SHOWING PIPE OF AIR-LIFT

"1. If the beds of clay, separating the polluted water in these strata from the deeper water, were or were not impermeable.

"2. If the methods of cutting off the upper strata by means of iron tubes and cement rings were or were not adequate.

"For this purpose one maintained that continuous pumping trials, extending over a period of 21 consecutive days, were essential so that a maximum depression might be obtained in the wells, which in this way would be subjected to a trial more severe than any they were likely to undergo when being pumped on for the town supply.

Extracts from final Report to the Sudan Government

"The condition of the wells which were tested in groups of two was as follows:—

"No. 1. Depth, 100 metres. Upper strata cut off to a depth of 65¾ metres.

"No. 5. Depth, 112 metres. Upper strata cut off to a depth of 69½ metres. Combined yield 18,000 gallons per hour.

"No. 6. Depth, 75 metres. Upper strata cut off to a depth of 53·82 metres.

"No. 7. Depth, 176 metres. Upper strata cut off to a depth of 72½ metres. Combined yield 21,000 gallons per hour."

It would take up too much space to give the results in detail. Their general consideration, as stated in the report, will suffice, but the tables in the Appendix will be found useful for reference (*vide pages* 314, 315 *and* 316).

"So far as Wells No. 1 and No. 7 go, these do not call for any lengthy discussion. A glance at the attached table will show that in both cases, and especially in No. 7, the colony count has almost throughout been uniformly low and compatible with that obtainable from true deep wells containing water free from bacterial impurity. On three occasions, March 18, 21 and 22, on which the count has exceeded 50 colonies per 1 c.c., the results can probably be adequately explained by the hypothesis that some of the foul condensation water from the air-pipe line was forced into the well. Personally, I am almost certain that this did occur on several occasions, and accounted for the greater number of colonies.* Excluding these abnormal counts, the average colony count for both wells works out at 5·8 per 1 c.c.

"Further, and this is of greater, indeed of paramount importance, *B. coli* has not once been found in either of these waters in the quantities (100 c.c. and under) examined, nor has any other objectionable organism been found.

"Hence, *at the present time*, the water of these two wells may be passed as a good drinking water, the only possible objection to it being that it is not well aerated and that it is warm, both faults which can be readily remedied.

"The slight odour of sulphuretted hydrogen in No. 7 Well-water does not call for any special criticism.

"Taking next Well No. 6, we find that its water closely resembles that found in No. 1 with a single exception. For the greater part of the trials there was present in it an organism closely allied to, if not identical with, *B. proteus fluorescens*.† It is necessary to consider the precise significance of an organism of this type in deep well-water. Its morphology and cultural characteristics will be found discussed in the Appendix, *page* 317, and, as the same organism was present in No. 5, the remarks here made will apply, so far as this matter is concerned, equally to that well-water. As has been pointed out *B. proteus fluorescens* is an organism capable of developing and multiplying in bile-salt media, and it is well known that the majority of organisms which do so are to be regarded with suspicion. Moreover, this is the very bacillus which Jaeger[15] and others have claimed, apparently with some reason, as the cause of infective jaundice (Weil's Disease), a condition not uncommon in Egypt and elsewhere in tropical or sub-tropical countries. It was, however, in Germany and the United States that the illness was found associated with the presence of this organism. It has, however, to be noted that in these cases the organism was isolated from *impure* water which had been drunk by the persons infected or in which they had bathed.

Presence of *B. proteus fluorescens*

"Now there is no evidence that the water in No. 6 Well is in any way contaminated by excretal products. The reverse is the case, for the standard indicator of impurity, *B. coli communis*, has never been found in it. While there are limits to the value to be attached to the chemical and bacteriological analyses of water, still a vast amount of most reliable evidence goes to show that the absence of *B. coli* from the water is the best known criterion of safety as regards water-borne diseases. This is the view I was inclined to adopt in the present instance, but as the matter was one of great interest and importance and as the organism has been proved pathogenic to man and was found to be highly virulent to certain rodents, I thought it well to lay the case before Dr. A. C. Houston, Director of Water Examinations to the Metropolitan Water Board, London, and probably the leading water bacteriologist of the present day. He has confirmed this view and states definitely that, although he has encountered chromogenic glucose (and sometimes lactose) fermenting microbes in well-water, he does not condemn the supply, other things being satisfactory and *B. coli* being absent. Acting on this principle, which appears to me eminently sound, I have passed No. 6 Well, more especially as the *B. proteus fluorescens* had disappeared from the water towards the end of the trials.

"Passing now to the consideration of Well No. 5, we find the problem is somewhat different. The water is clearly not derived from precisely the same source as in the case of the other wells. This is evident from its uniformly lower temperature, which no consideration of the pipe arrangements, etc., will explain. Moreover, the bacteriological content differs to some extent. The colony count was more variable and not quite so satisfactory. Chemically, also, there are slight, but possibly significant, differences (*vide* Dr. Beam's report, *page* 312). Thus, although *B. coli* has never been found in this water, the facts stated, together with the constant presence

* It is interesting that the air blown into the water did not in any way seem to affect the colony count. At first it was carefully filtered, but afterwards this precaution was abandoned, for observations made by Mr. Williams showed that the 500 cubic feet of free air passing per minute through the cylinders was probably subjected to a temperature of 220° F. This, though but momentary, would, I think, serve effectually to sterilise it.

† This bacillus was present as a rule in 5 c.c. and larger quantities but was not infrequently found in 1 c.c. of the water.



G. Buchanan

Fig. 100.—Large Covered Reservoir
By its use the quantity of Iron present in the supply to the Town has been considerably reduced



G. Buchanan

Fig. 101.—Bornes Fontaines, showing faulty method of distributing water in canvas sacks slung over donkeys

of *B. proteus fluorescens*, led one to regard this source of supply with some suspicion. I think it would be advisable not to pass No. 5 until we have learned more about it. The water would appear to come from nearer the surface. It does not seem to have any immediate connection with the river, and as the yield is small, would appear to be derived from some bed of limited extent which perhaps trends towards the surface. This is not an easy question to settle, and of equal difficulty is the determination of the source of *B. proteus fluorescens* both in Well No. 5 and Well No. 6. It appears that adjacent to the water works there is a bed of decomposing vegetable material close to the southern river bank and about a metre under the river bed. This was encountered when the pipe trenches for the condensation water from the Blue Nile were dug, but no information was furnished me on this point and I only learned it recently from one of the officials. Considering, however, that the cutting-off by cement is satisfactory, and that the deep clay beds appear to be quite impermeable, it would not seem that any contamination could be derived from this source, and in any case such contamination might not be of a dangerous nature. The suggestion has been made that the cement used in the cutting-off process might have become contaminated from the feet of the native workpeople, and that the water-bearing beds had thus been rendered temporarily impure. If this were so, however, one would have expected a much higher colony count and a much greater variety of microbes while some type of *B. coli* would almost certainly have been present, unless, indeed, other organisms, apart from *B. proteus fluorescens*, had died out in the pure, deep water. This is not very likely, as certain varieties of *B. coli* are known to be fairly resistant. The organism does not appear to have come from the air-pipe line.

Doubt as to Well No. 5

"It would serve no purpose to discuss this matter at greater length, but there is one point on which special stress must be laid. *The results obtained can only be considered applicable to the wells under existing conditions.* True, the tests have been fairly exhaustive and severe, and, personally, I think it likely that the water in Wells Nos. 1, 6 and 7 will continue to be a safe source of supply, but one cannot be absolutely certain, for it is possible, though not probable, that changes in the river may induce changes in the deep water. Hence the necessity for routine bacteriological tests, which, indeed, are nowadays carried out in connection with all well-conducted town supplies. These will be combined with occasional chemical examinations, and this leads one to draw attention to the attached report from Dr. Beam.* On this occasion, chemical tests are of subsidiary importance, but they possess considerable interest, and, so far as they go, they confirm the bacterioscopic analyses.

"There is nothing in them to lead to a condemnation of any of the well-waters. The quantities of iron and manganese present are too low to be likely to cause any serious trouble with crenothrix growth, provided the large storage tank, now in course of construction, is in use. At the same time they are undesirable ingredients, though it will be noted that with the exception of Well No. 5, they tended to diminish as pumping proceeded. It will be also noted that the plumbo-solvent power of the water was tested, although very little lead piping will be used in Khartoum.

The following conclusions, therefore, appear justifiable:—

"1. From a chemical standpoint the four wells tested, Nos. 1, 5, 6 and 7 have yielded a satisfactory water.

"2. Bacteriologically, Wells Nos. 1, 6 and 7 have yielded a satisfactory supply.

"3. Bacteriologically, Well No. 5 has not proved so satisfactory as the others, and is not passed at present. As a matter of fact, I understand that as there is a low yield from this well the Director of Works has no particular objection to its being closed, and this may possibly be done.

"4. The examinations made refer only to the water under existing conditions, though it is believed that the tests have been sufficiently prolonged and severe to make it probable that the supply will remain satisfactory as the source itself seems to be above suspicion.

"5. It will be necessary to conduct routine bacteriological examinations and occasional chemical tests while the conditions of storage will require careful consideration.

"6. The question of quantity is one for engineers to settle, but, from the data furnished, there would appear to be an ample supply, at least, for the present."

Conclusions justified

I may say these conclusions have so far been amply justified. For a year and more routine tests have been made, the Nile has risen, fallen and risen again, the well-water has been in general use, the quality has remained good, and apart from a little trouble in the pipes owing to crenothrix, the supply has been quite satisfactory. A large covered reservoir has now been constructed and this will tend to improve matters so far as iron is concerned (Fig. 100).

A paper of this nature should, perhaps, deal with questions of distribution, storage, and methods of water sterilisation in the Tropics, but, owing to lack of local experience in these directions, such additions would be more or less of the nature of a compilation, and, as they are dealt with in our Second Review, may be omitted, save for some reference to distribution methods in the Appendix. Therefore, I would only again lay stress on the demonstration which has been afforded us of the great value of *B. coli*, even Houston's old, merely partially qualified, elastic *B. coli*, as a standard of pollution under certain conditions in the Tropics. It would have been most interesting to apply Clemesha's tests to the *B. coli* which was found in the deep well-water, but his paper had not been published, and in any case, time would not have admitted of such a research. I wonder, however,

* For details of the chemical analyses, and full comments upon them, see Dr. Beam's report, Volume B., p. 26

into which of Clemesha's groups the *B. coli* recently found by Thresh[16] in deep well-waters in England would go? In Khartoum, Clemesha's distinctions were, for several reasons, unnecessary, but it is possible they may be of value even for deep well-waters, and not only in the Tropics, although, considering the basis on which they rest, this is, at least, doubtful. In any case, I think it will be admitted that the subject is one of great interest, while, apart from the vexed question of *B. coli*, I think some of the problems mentioned, some of the results recorded, and some of the conclusions reached, may be of service to those who have the responsibility of examining and reporting upon town water-supplies in tropical countries.

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Preliminary Tests

WELL No. 6

Date	Strata cut off by cement	Average colony count per 1 c.c.	*B. coli* "Flaginac"	Chromogenic sugar-fermenting bacillus
December 17, /08	+	24	—	—
February 9, /09	+	6	—	—
February 22, /09	+	6	—	—
February 23, /09	+	2·3	—	—
February 24, /09	+	3	—	—

WELL No. 7

December 12, /08	—	38	No examination	No examination
January 14, /09	—	55	+	—
March 4, /09...	+	33	—	—

Special Tests

21 days' pumping trials

WELL No. 1

Temperature of water varied between 29·8 and 30·2° C.

Date	Strata cut off by cement	Average colony count per 1 c.c.	*B. coli* "Flaginac"	Chromogenic sugar-fermenting bacillus
March 4, /09...	+	33 ? sampling unsatisfactory	—	—
,, 9, /09...	+	4	—	—
,, 10, /09...	+	?	—	—
,, 11, /09...	+	10	—	—
,, 12, /09...	+	?	—	—
,, 13, /09...	+	?	—	—
,, 15, /09...	+	?	—	—
,, 16, /09...	+	?	—	—
,, 18, /09...	+	31	—	—
,, 20, /09...	+	?	—	—
,, 21, /09...	+	91	—	—
,, 22, /09...	+	137	—	—
,, 23, /09...	+	2	—	—
,, 25, /09...	+	11	—	—
,, 26, /09...	+	2·6	—	—
,, 27, /09...	+	5	—	—
,, 28, /09...	+	5·2	—	—

WELL No. 5

Temperature of water varied between 27·5 and 27·9° C. usually was 27·8° C.

Date	Strata cut off by cement	Average colony count per 1 c.c.	*B. coli* "Flaginac"	Chromogenic sugar-fermenting bacillus
March 9, /09...	+	74	—	+
,, 11, /09...	+	10	—	+
,, 12, /09...	+	?	—	+
,, 13, /09...	+	?	—	+
,, 14, /09...	+	?	—	+
,, 15, /09...	+	?	—	+
,, 18, /09...	+	101	—	+
,, 20, /09...	+	?	—	+
,, 21, /09...	+	77	—	+
,, 22, /09...	+	26	—	+
,, 23, /09...	+	8	—	+
,, 25, /09...	+	11	—	+
,, 26, /09...	+	15	—	+
,, 27, /09...	+	15	—	+
,, 28, /09...	+	27	—	+

WELL No. 6

Temperature of water varied between 29·5 and 30° C.

Date	Strata cut off by cement	Average colony count per 1 c.c.	*B. coli* "Flaginac"	Chromogenic sugar-fermenting bacillus
April 3, /09...	+	9	—	+
,, 5, /09...	+	4·4	—	+
,, 7, /09...	+	?	—	+
,, 9, /09...	+	?	—	+
,, 11, /09...	+	11	—	—
,, 13, /09...	+	25	—	+
,, 15, /09...	+	10·3	—	—
,, 17, /09...	+	113	—	+
,, 19, /09...	+	10	—	—
,, 21, /09...	+	5	—	—
,, 22, /09...	+	4	—	—
,, 23, /09...	+	11	—	—

WELL No. 7

Temperature of water varied between 30·1 and 30·7° C. usually was 30·4° C.

Date	Strata cut off by cement	Average colony count per 1 c.c.	*B. coli* "Flaginac"	Chromogenic sugar-fermenting bacillus
April 3, /09	+	9	—	—
,, 5, /09	+	5	—	—
,, 7, /09	+	?	—	—
,, 9, /09	+	?	—	—
,, 11, /09	+	7	—	—
,, 13, /09	+	7·4	—	—
,, 15, /09	+	7·5	—	—
,, 17, /09	+	5·5	—	—
,, 19, /09	+	9	—	—
,, 21, /09	+	5	—	—
,, 22, /09	+	2·4	—	—
,, 23, /09	+	3	—	—

Note.—In all, some 250 separate counts and 63 qualitative examinations were made up to this date (April 23, 1909).

Appendix

From April, 1909, to the end of 1910, the following counts have been conducted and the following results obtained:—

Well No. 1. 4 counts. Average colony count=7·5 per 1 c.c.

Well No. 6. 34 counts (2 false and discarded).

Average colony count=5 per 1 c.c.

Well No. 7. 30 counts (5 false and discarded).

Average colony count=7 per 1 c.c.

A false count means one where there was evidently contamination from the pipe-line, causing a marked but transitory increase in the number of colonies.

It will be seen that the counts from the three are much the same, as was indeed to be expected, and that the water, from the point of view of the number of micro-organisms present in it, is of exceptionally good quality, answering to that usually derived from true deep wells.

Notes on Distribution

Distribution

Methods of distribution are, in the main, engineering questions, and in the case of the Khartoum water-supply I only propose to consider the method employed in so far as it has a hygienic bearing.

The system adopted, and carried through without any reference to those responsible for the public health, is that known as the "Dead-end." From the sanitary standpoint the disadvantages of this system are that the water is liable to remain stagnant for indefinite periods in the "dead-ends" of the pipes with the result that it deteriorates in quality. These disadvantages are increased when, as in the case under discussion, the water contains an excess of iron, for its stagnation favours the development of the crenothrix fungus as well as of any deleterious or objectionable organisms which may happen to be present. Although the chromogenic, lactose-fermenting bacillus mentioned

in the report (*page* 310) is not hurtful and does not appear to taint the water, under any known conditions, its multiplication, as shown by bacteriological examination, is favoured by this "dead-end" system.

When it cannot be detected in small quantities of water taken at the wells it can be easily found in similar amounts taken from taps at various points on the pipe system (*vide infra*). At the same time its presence has no special significance although one would be better pleased if it were absent altogether.

Stagnation in "dead-ends." How dealt with

The stagnation can be overcome by occasionally flushing out the pipes by hydrants at the "dead-ends," but naturally this involves a waste of water, especially when it has to be frequently done to clear the pipe system of crenothrix growth, discoloured water or water which, if left alone, might possibly acquire an unpleasant smell or taste from the presence of the gas-forming bacillus. It is, however, only fair to say that laboratory experiments on a small scale have not shown any signs of risk in this last direction. One gives here the morphological characteristics and pathological effects of the organism in question. These were not fully discussed in the paper.

It was found to be a short, motile rod, Gram-negative, but staining readily by the ordinary aniline dyes. Its chief cultural characteristics were as follows:—

On agar slope it produced slimy, grey colonies with thin bluish-green margins, and it soon coloured the medium a bright green colour. The colonies had a bad smell similar to those of the proteus group. Neutral red was reduced with a yellow fluorescence, gas, but not in large amount, was produced in a glucose medium, and certain strains, also fermented lactose; but this was not a constant characteristic. In liquid media, a thick scum was produced. The colonies on Drigalski-Conradi were thick, slimy, dark greenish-blue and evil-smelling; on Endo they were pinkish-red—not unlike those of *B. coli*, but spreading with a beautiful violet fluorescence; on lactose bile-salt agar they were, as a rule, a greenish-blue with well marked fluorescence.

Indol was produced in appropriate media, while in litmus whey the colour was, as a rule, discharged. The effect on litmus milk varied. Sometimes the medium remained unchanged, sometimes the milk clotted and was afterwards liquefied, sometimes considerable alkali was produced. On potato a dull slimy brown growth developed, and the potato was coloured a deep green. Blood serum was rapidly digested.

The organism was not pathogenic for guinea-pigs or fowls, but killed jerboas and gerbils speedily, both when injected subcutaneously and intraperitoneally.

Bacteriological examinations of tap waters

Appended will be found an account of the bacteriological examinations conducted on tap waters in different parts of the town. It will be seen that there is always a considerable multiplication in the number of colonies and that some form of gas-producing organism—usually the green lactose fermenter—is almost invariably present in the quantities examined.

Eleven examinations of tap water in Khartoum were made between May, 1909, and January, 1910. The average colony count was 50 per 1 c.c. The locality did not seem to influence the count. There were fewer colonies in the Grand Hotel water than in the Palace water which is only about half as far from the source of supply.

In eight instances the chromogenic, lactose-fermenting bacillus was found. In one instance an atypical *Bacillus coli* was found.

In one instance a non-chromogenic, lactose-fermenting, proteus-like organism was found. In the remaining case no gas-forming, sugar-fermenting organism was found.

At present local distribution is carried out in two ways. In the first and second class quarters of the town, meters have been installed and the water is laid on to the

individual houses. In the third class quarters, *Bornes Fontaines* were erected. These, while quite good in themselves, were not sufficiently numerous, and necessitated the presence of attendants to sell the water and the use of boys as water-carriers, who distributed the water in canvas sacks, slung over donkeys (Fig. 101, *page* 311).

It is evident that such an arrangement presented elements of danger. A pure supply ran great risk of fouling, for the donkey-boy's hands are not over clean, neither are his sacks, neither is his donkey, while he is apt to stuff up the inlet to his sacks with anything which comes handy, usually a bit of filthy rag, and occasionally even bestrides the bags. There was also waste of water, and puddles formed which were churned into mud.

Distribution

It was agreed to abolish the *Bornes Fontaines*, and to substitute for them a sufficiency of stand-pipes with self-closing valves, allowing the people to take as much water as they required and charging a water-rate calculated on rental. I regret to say that money for this scheme has not yet (January, 1911) been sanctioned, despite repeated representations. Happily, the faulty conditions prevailing have not, so far as known, caused any ill-effects to the health of the population, but the elements of danger are present, and it is imperative that the necessary funds be forthcoming without delay.

Pending the settlement of the question, it has not been possible to substitute water-carts for the donkey-bags, but had one known that there would have been such delay in arranging a vital matter of this kind, one would have insisted on the provision of proper water-carts in the face of financial objections. Now one can only play the importunate widow, a course of action which, in public health matters in this country, usually has the desired effect within a reasonable time.

The Presence, Type, and Possible Significance of Lactose-Fermenting Bacilli in Surface Waters and in the Fæces of Man and certain Domestic Animals in the Sudan together with a consideration of the effects of Sunlight and Earth Burial on these Organisms

Preliminary Note

by

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It is still more or less an open question, whether the standards of purity for water-supplies set down by sanitarians in England and America should be adopted in tropical towns and districts. The conditions which in the Tropics influence, more or less, the purity of a water-supply are often so different from those obtaining in temperate climates that it is still a doubtful point whether the strict standards which are adopted in England are at all applicable to tropical climes where the water-supply is often derived from shallow wells and rivers exposed to a considerable amount of fæcal contamination of both human and animal origin. In England, the dangerous pollution that is most common in rivers has its origin chiefly in sewage from large towns, and that form of pollution is mainly derived from man.

Standards of purity

If samples of water taken from shallow wells or rivers in the Tropics are subjected to a few simple tests for the presence of fæcal contamination, the results will often show such a state of things that no analyst in England would ever consider the passing of such water as fit for human consumption, and yet the water from these sources is used daily by both Europeans and natives alike without any ill-effects to health so far as can be told.* The question naturally arises, whether, in the face of these existing conditions, one would be justified in using the European standards of water purity as a guide, or whether some modification of the European standard could be generally employed in tropical climes.

Recently, Clemesha, Aiyar and Mudaliyar,[1] carried out a series of extensive tests on a large number of water-supplies in India. Their researches were of an elaborate nature; not only did they conduct the ordinary bacteriological and chemical examinations of these water-supplies, but they also tested the effects of sunlight and storage on the various groups of organisms isolated by them. Their experiments were of such a practical nature, that it would be well if sanitarians in other countries would take up similar lines of research, and the results obtained might materially assist the medical officer of health in the Tropics in arriving at some conclusion as to the purity or otherwise of the water-supplies for which he is responsible.

Observations on Indian water-supplies

* The proviso is necessary as the correctness of the Mills-Reincke phenomenon may yet be proved. (*Vide Journal Infectious Diseases*, August 24, 1910.)

[1] Clemesha, W., Major, Aiyar, T. S., and Mudaliyar, V. G. (1908), "A study of the bacteriology of drinking-water supplies in tropical climates." *Annual Report of the King Institute of Preventive Medicine*, Madras, for 1908.

In order to see if the conditions described by Clemesha, Aiyar and Mudaliyar* were comparable with those existing in the Sudan, a series of a few experiments, commenced some months ago, was carried out, and they form the subject of a paper which cannot be considered as at all complete owing to the fact that they were too few in number and only represented a condition of things existing for three months in Khartoum.

They serve, however, as an introduction to a more prolonged research on the subject of the types of fæcal organisms present in water and soil and the effects of sunlight, etc., on such organisms as have been isolated.

Khartoum water-supply

It may be as well to state here the nature of the water-supplies present in Khartoum. Up till two years ago the town was dependent for the most part on the water taken from the banks of the Blue Nile. Various shallow wells were present in the town, but the native population, as a rule, only used the water from this source for cooking and washing purposes. Now, however, a different state of things exists as most of the shallow wells have been put out of use, and the general water-supply is obtained from deep wells near the Blue Nile. The water obtained from these deep wells is now of great purity both chemically and bacteriologically, as proved by the large number of tests that have been carried out on it at all times of the year.

The climate of Khartoum is on the whole an extremely dry one. July and August are the chief rainy months. April to June represent the hottest and most dusty months. In September and October the weather is hot and the atmosphere comparatively humid. The Nile as a rule commences to rise at Khartoum in June and reaches its maximum height in September. In November, December, January and February the weather is dry and cool.

Technique

In carrying out the bacteriological analyses of the various water samples the technique employed was more or less the one used by Clemesha. About 250 c.c. of the sample were collected in a sterile bottle and the latter was surrounded by ice so as to prevent multiplication of the organisms present. After removal to the laboratory the sample was subjected to the following tests: (1) estimation of the number of colonies present in 1 c.c. of the sample; (2) *enteritidis sporogenes* milk test in 20 c.c. of water; (3) the presence of lactose fermenters in various quantities of water. Before carrying out these tests the sample was always well shaken.

1. *The total colony count in* 1 *c.c.*

This was always carried out with agar plates as the heat prevented the use of gelatine. The percentages of the various constituents of the agar was the same throughout all the tests. The acidity was + 10 to phenol-phthalein. One c.c., ·5 c.c. and ·2 c.c. of the water sample is taken and run into melted agar of a temperature of 41° C. This is then plated out in Petri dishes, incubated at 37° C. for 48 hours, and all colonies visible to the unaided eye are counted.

2. *Sporogenes test*

Twenty c.c. of the sample water are put into sterile milk contained in tubes. The milk is then heated up to 80° C. for 20 minutes, and a layer of hot vaseline is run on to the surface of the milk so as to render the latter anærobic. The milk tubes are then incubated at 37° C. for 48 hours.

Evidence of the presence of the spores of *B. enteritidis sporogenes* is shown by the clotting of the milk.

3. *The test for the presence of lactose-fermenting organisms and their subsequent isolation*

Lactose bile-salt-neutral-red-broth is used and, as varying quantities of water are inoculated into the tubes containing this medium, the latter is made up in single, double

* See general account beginning on *page* 333

and triple strengths. One tube is inoculated with 20 c.c. of the water; two tubes with 10 c.c., 4 tubes with 5 c.c., 5 tubes with 1 c.c., 5 tubes with ·1 c.c., and 5 tubes with ·01 c.c. All are incubated at 37° C., only tubes that give both acid and gas being counted as having given the reaction. If only one tube in the first seven of these gives a reaction, then fæcal bacilli are present in 60 c.c., and if three out of the five 1 c.c tubes give a positive reaction, then fæcal bacilli are considered to be present in 1 c.c. The same applies more or less to the ·1 c.c. and ·01 c.c. groups. The next step in the procedure is to isolate the various fæcal organisms present; and in order to do this satisfactorily, the tube containing 10 c.c. of water or more, that has given a positive reaction in 18 hours, is selected.

It is an important point that a tube containing at least 10 c.c. of the water sample and giving the positive reaction within 18 hours, be selected. If tubes containing a smaller amount than 10 c.c. of the water sample be selected, the danger is that only one or two fæcal bacilli may be present, and, if a tube be selected for subculturing purposes which has been longer in the incubator than 18 hours, the chances are that many of the lactose fermenters have had their growth inhibited by the increasing acidity of the medium.

Isolation of fæcal organisms by plating

Having selected then a tube containing at least 10 c.c. of the water sample, which has been incubated for not more than 18 hours, three to five platinum loopfuls are removed and inoculated into 10 c.c. of sterile water. The latter is then vigorously shaken, and one loopful of this mixture is then plated out on lactose bile-salt-neutral-red-agar, which is incubated at 37° C., for 48 hours. At the end of that time 15 to 20 colonies are found to be present in the plate. A segment of the plate is marked off if more than this number is present. Ten colonies are in any case picked off, and each single colony is inoculated into a tube containing a few drops of sterile water. From these tubes further inoculations are made into broth, glucose-peptone, and four different, special sugars and incubated at 37° C. The inoculated broth tube is examined at the end of 18 hours for motility and the production of indol. The glucose-peptone is incubated for 48 hours and, at the end of that time, if a strong growth is present, a few drops of a very concentrated solution of caustic potash are added for the purpose of finding if the Voges and Proskauer reaction is given. If this is present, a bright orange-red colour is produced. It may take 24 hours before this colour is apparent.

Differential sugar media

The four sugars that are inoculated are those recommended by MacConkey,[1] namely saccharose, dulcit, adonit and inulin. In these, a positive reaction is represented at the end of 48 hours incubation at 37° C. by the production of acid and gas. In carrying out these tests the problem of the constancy of the reaction of the organisms in the various sugars was not taken into account, it being accepted as a fact that the same species of bacillus always fermented the same sugar.

In connection with the indol test the paradimethyl benzaldehyde reaction was always used, as it seems to be a very delicate and reliable one.

With regard to the Voges-Proskauer reaction, considerable difference of opinion exists, but there can be but little doubt that it appears a good test for the *B. cloacæ*, *B. lactis ærogenes* and *Oxytocus perniciosus* group.*

As will be seen from the results of the following examinations, a large number of the organisms will be found to be non-motile. The question of motility, as a means of differentiating an organism of the coli group should not, in the opinion of the writer, be regarded seriously as compared with the reactions in the sugars and the indol test.

It may be stated here that in order to simplify the nomenclature of the fæcal

[1] MacConkey, A. (July, 1905), "Lactose fermenting bacteria in fæces." *Journal of Hygiene.*

* *Vide page* 326

organisms met with in these experiments, the term *B. coli* was only applied to Escherich's organisms. All bacilli giving different reactions in any permanent test were looked upon as different species, and different names and numbers were given to them.

Further, by using the two sugars, saccharose and dulcit, one was able to adopt MacConkey's method of dividing all lactose fermenters into the following four groups:—

Group I Ferments neither saccharose nor dulcit
„ II „ dulcit but not saccharose
„ III „ dulcit and saccharose
„ IV „ saccharose but not dulcit

The following few experiments were carried out to estimate the varieties of fæcal organisms present in river and well-water, etc. Numbers were given to organisms that did not correspond in their reactions to any named bacillus, and, as mentioned, the term *B. coli* was applied only to Escherich's organism.

In addition to being put into the media described above, the organisms isolated were also inoculated into glucose, litmus milk, and inosit, and although the results obtained with these media do not figure in the tables, the reactions were made use of in attempting to identify any doubtful organism.

The sign + indicates the formation of acid and gas.

The sign − indicates a negative reaction as regards the presence of acid and gas, and an asterisk indicates that the bacillus is an unnamed one.

Experiment I.—Date, July 16, 1910. Local rainfall, nil.

Sample taken from the Blue Nile at a distance of two metres from the river bank.

The river had been rising steadily for a period of three weeks owing to the rainfall up-country.

Temperature of water, 27° C.

(*a*) Total colonies on agar at 37° = 700 per 1 c.c.

(*b*) Fæcal bacilli present in ·5 c.c.

(*c*) Sporogenes milk test + in 20 c.c.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
1	+	—	+	—	+	—	—	—	+	II	*B. coli*
2	—	—	+	—	+	+	—	—	+	II	*
3	—	—	+	—	+	+	—	—	+	II	*
4	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
5	+	—	+	—	+	—	—	—	+	II	*B. coli*
6	+	—	+	—	+	—	—	—	+	II	*B. coli*
7	+	—	+	+	+	—	—	—	+	III	*
8	—	—	+	—	—	—	—	—	+	I	*B. vesiculosus*
9	+	—	+	—	+	—	—	—	+	II	*B. coli*
10	+	—	+	—	+	—	—	—	+	II	*B. coli*

Group I, 1 Group II, 7 Group III, 2

Experiment II.—Date, November 24, 1910. Local rainfall, nil.

Sample taken from the Blue Nile two metres from the river bank. The river had been steadily decreasing in volume for a period of two months. Temperature 24·5° C.

(*a*) Total colonies on agar at 37° C.=218 per 1 c.c.

(*b*) Fæcal bacilli present in ·1 c.c.

(*c*) Sporogenes milk test + in 20 c.c.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
51	+	—	+	—	+	—	—	—	+	II	*B. coli*
52	+	—	+	—	+	—	—	—	+	II	*B. coli*
53	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
54	—	—	+	—	—	—	—	—	—	I	*B. coli mutabilis (Massini)*
56	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
57	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
58	+	—	+	+	+	—	—	+	+	III	*
59	—	—	+	—	—	—	—	—	+	I	*B. vesiculosus*
60	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*

Group I, 2 Group II, 2 Group III, 5

Experiment III.—Date, July 28, 1910. Local rainfall, nil.

Sample taken from a shallow well situated in a native hoosh (yard) about a quarter of a mile from the Blue Nile.

The water was at a depth of 19 metres from the surface. Temperature 30° C.

(*a*) Total colony count in agar at 37° C. = 700 per c.c.

(*b*) Fæcal bacilli present in ·01 c.c.

(*c*) Sporogenes milk test + in 20 c.c.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
31	—	—	+	+	—	+	—	+	—	IV	*B. lactis ærogenes*
32	+	—	+	—	—	—	—	—	+	I	*
33	—	—	+	+	—	+	—	+	—	IV	*B. lactis ærogenes*
34	—	—	+	—	—	—	—	—	—	I	*B. coli mutabilis (Massini)*
35	—?	—	+	+	—	—	—	—	—	IV	*
36	—	—	+	+	—	—	—	—	+	IV	*B. coscoroba*
37	+	—	+	—	—	—	—	—	+	I	*
38	—	—	+	+	—	+	—	—	+	IV	*
39	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
40	—	—	+	+	+	+	—	—	+	III	*

Group I, 3 Group II, nil Group III, 2 Group IV, 5

* About all these there is a certain amount of doubt

Experiment IV.—Date, July 28, 1910. Local rainfall, nil.

Sample taken from Well No. 7 of the Khartoum water-supply.

Gas was present in MacConkey's media when the latter was inoculated with 50 c.c. of the sample.

Sporogenes milk test negative in 20 c.c.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
11	+	—	+	+	+	—	—	—	+	III	*
12	+	—	+	+	+	—	—	—	+	III	*
13	+	—	—	+	+	—	—	—	+	...	*
14	+	—	—	+	+	—	—	—	+	...	*
15	+	—	—	+	+	—	—	—	+	...	*
16	+	—	—	+	+	—	—	—	+	...	*
17	+	—	—	+	+	—	—	—	+	...	*
18	+	—	—	+	+	—	—	—	+	...	*
19	+	—	—	+	+	—	—	—	+	...	*
20	+	—	+	+	—	—	—	—	+	IV	*

Group I, nil Group II, nil Group III, 2 Group IV, 1

Conclusions

One may here state that the observations have, so far, been too few in number to justify any definite conclusions being arrived at with regard to the position that the *B. coli* occupies in this country as an indicator of recent pollution. Still, some of the experiments show interesting points as regards the vitality of this organism in nature. In order to make all these experiments as complete as possible, tests require to be carried out during every month in the year, and it would thus be possible fully to determine the effects of external natural forces such as sunshine and rain on all organisms of fæcal origin. All such research would afford valuable information. The drawback in carrying it out is the large amount of media that is required, and in laboratories such as these, where work of another nature requires constant attention, the time necessary to carry them out completely is not available.

Pollution greater with falling than with rising Nile

Experiments I. and II. were of interest, as they illustrated that a greater degree of fæcal pollution was present in a "falling" than in a "rising" river, although the colony count in the latter was nearly four times as great as in the former. There was a considerable difference in the lowest "end" points in which fæcal bacilli were obtained.

Out of ten colonies of lactose fermenters present in (1) the rising Nile there were:—*B. coli* = 5, *B. neapolitanus* = 1, *B. vesiculosus* = 1, and three others not identified.

(2) Falling Nile.

B. coli = 2, *B. neapolitanus* = 4, *B. vesiculosus* = 1, *B. coli mutabilis* = 1, and two others not identified.

Group IV. was absent in both samples.

Experiment III. was carried out to determine the types of lactose fermenters present in a shallow well situated in a native hoosh, and represented the type common in Khartoum. The degree of pollution present was considerable. The types of lactose fermenters present were, out of ten test colonies:—*B. lactis ærogenes* = 3, *B. coscoroba* = 1, *B. neapolitanus* = 1, *B. coli mutabilis* (*Massini*) = 1, and four others not identified.

ADDITIONAL EXPERIMENTS. *(Vide* Tables, *page* 327*)*

A. Lactose fermenters in human fæces

Experiments V. and VI. were carried out for the purpose of ascertaining the types of lactose fermenters present in human fæces. Experiment V. was carried out in the month of August. Experiment VI. three months later. One gramme of fæces was taken and added to a tube of MacConkey's broth, and the latter well shaken up so as to get as good an emulsion as possible. The tube was incubated then for 24 hours at 37° C. and five loopfuls were removed and inoculated into a tube of sterile water, from which subcultures were made on lactose bile-salt-neutral-red-agar plates. The latter were incubated at 37° C. for 48 hours, and, as usual, ten colonies picked off and their reactions tested.

Seasonal variation of lactose fermenters in human fæces

Reference to the tables will show that these two experiments illustrate the fact that different types of lactose fermenters are apparently present in human fæces in different months of the year and, if this is true, in order to get the fact more fully established, such experiments will require to be carried out throughout the year.

B. Effect of sunlight on lactose fermenters in human fæces

It is a well-recognised fact that sunlight has a powerful effect on different micro-organisms, and there can be no doubt that the sun's rays in the Tropics play an important part as a bactericidal agent.

Some rough experiments were carried out to illustrate this, and more especially with reference to the *B. coli*. Laboratory cultures were not employed, as, apart from the fact that they did not represent the condition of things in nature, Clemesha in India obtained irregular results by using them.

The procedure adopted was as follows:—

One gramme of human fæces was made into an emulsion with one litre of sterile water and well mixed. Five c.c. of this mixture was removed and inoculated into MacConkey's bile-salt broth in order to determine the types of lactose fermenters present, and the remainder was put into a glass jar covered with a glass lid, and exposed to the sun's rays. Samples of 5 c.c. were removed at intervals and plated out to determine the types of organisms present. Before removing these samples, the jar was well shaken. The temperature of this emulsion varied from 26° C. to 41° C. The depth of the emulsion was not kept constant. The results of this experiment were as follows:—

Effect of sunlight

Before exposure

Organisms present in emulsion. In ten lactose fermenters *B. coli* = 1, *B. neapolitanus* = 4, *B. vesiculosus* = 1, and four organisms not identified.

After exposure to sunlight for 24 *hours*

B. coli = nil, *B. neapolitanus* = 6, *B. schæfferi* = 1, and three organisms not identified.

After exposure to sunlight for 48 *hours*

B. coscoroba = 2, and seven others of the *B. neapolitanus* type.

After exposure to sunlight for 6 *days*

Organisms belonging to Groups I. and IV. were present.

After exposure to sunlight for 10 *days*

B. schæfferi = 4, *B. neapolitanus* = 6

After exposure to sunlight for 30 *days*

B. coscoroba = 2, *B. schæfferi* = 4, *B. neapolitanus* = 3, *B. vesiculosus* = 1.

The results of these experiments tend to show that *B. coli communis* is extremely susceptible to the bactericidal effects of sunlight, and that *B. coscoroba*, *B. schæfferi*, *B. neapolitanus* and *B. vesiculosus* are extremely resistant. The average period of sunshine per diem during these experiments was $11\frac{1}{2}$ hours.

Experiments were also carried out for the purpose of ascertaining the bactericidal effects of the sun on all fæcal organisms. Small quantities of the emulsion, *i.e.* 1 c.c., were removed at intervals and plated out on lactose bile-salt-neutral-red-agar and incubated for 48 hours at 27° C.

Before exposure to sunlight		= colonies too numerous to count
After	1 hour	= colonies too numerous to count
,,	6 hours	= between 800 and 900
,,	24 hours	= between 200 and 300

A large number of experiments must still be carried out before it will be possible to classify the organisms in this country according to the effects of sunlight on them. But this will be possible if work be conducted on the lines suggested by Clemesha, and the results of such research will aid considerably in adopting a bacteriological standard for drinking-water from surface sources.

C. Effect of earth burial on the bacterial content of human excrement

Effect of soil

One other important factor in nature, closely associated with the bacteriology of water, has also to be considered, and that is the effect of soil on the bacteria in human excrement and the way in which nature gets rid of soil pollution of this kind. In order to study such effects, samples were taken from the trenching grounds where all the sewage of the town is pitted. The soil is of a loamy nature and the excreta are buried in pits 18 to 24 inches deep. Samples were removed from a depth of 4 inches, and one gramme placed in MacConkey's bile-salt broth and incubated for 24 hours at 37° C. Subcultures were taken and plated out on lactose bile-salt-neutral-red-agar and various colonies picked off and put into the different media.

The experiments showed that *B. coli*, though present in 24 hours, was not found in the contents of trenches 7 days old, and that such sun-resisting organisms as *B. schæfferi*, *B. neapolitanus* and *B. vesiculosus* were still present in trenches 14 days old.

D. Lactose fermenters in animal excreta

Lactose fermenters in Goat's fæces

In this country the part played by animal pollution of water-supplies cannot be overlooked. The commonest animal owned by the native in the Sudan is the goat. The types of organisms met with in the fæces of the goat were *B. coli*, *B. cloacæ*, *B. neapolitanus* and others which have not been identified.

In the fæces of the cow *B. neapolitanus* and *B. schæfferi* were present, and there were several that gave a positive Voges-Proskauer reaction. According to MacConkey, these latter organisms are closely associated with the *B. lactis ærogenes* and *B. cloacæ* group.

No conclusions as regards the effect of sunlight and soil on the fæcal organisms of these animals have yet been arrived at as further experiments are in process of being carried out.

The lack of completeness in these experiments prevents any definite conclusions being stated as regards the adoption of a bacteriological standard for drinking-water derived from surface sources in this country. As mentioned above, they only represent a preliminary introduction to an extensive research that is being carried out on a subject of great importance.

ADDITIONAL EXPERIMENTS

Date, August 1, 1910

To determine the types of lactose fermenters present in human fæces.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
21	+	—	+	+	—	—	—	—	+	IV	*
22	+	—	+	—	+	+	—	—	+	II	*
23	+	—	+	—	+	—	—	—	+	II	*B. coli*
24	+	—	+	—	—	—	—	—	+	I	*
25	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
26	+	—	+	+	+	—	—	—	+	III	*
27	—	—	+	—	—	—	—	—	+	I	*B. vesiculosus*
28	+	—	+	+	+	—	—	—	+	III	*
29	+	—	+	+	+	—	—	—	+	III	*
30	+	—	+	+	+	+	—	—	+	III	*

Group I, 2 Group II, 3 Group III, 4 Group IV, 1

Date, November 30, 1910

To determine the types of lactose fermenters present in human fæces and the effects of sunlight on them.

A. Before exposure to sunlight

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
61	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
62	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
63	+	—	+	+	—	—	—	—	+	IV	*
64	—	—	+	—	—	—	—	—	+	I	*B. vesiculosus*
65	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
66	+	—	+	+	—	—	—	—	+	IV	*
67	+	—	+	+	+	—	—	—	+	III	*
68	+	—	+	—	+	—	—	—	+	II	*B. coli*
69	+	—	+	+	—	—	—	—	+	IV	*
70	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*

Group I, 1 Group II, 1 Group III, 5 Group IV, 3

* About all these there is a certain amount of doubt

ADDITIONAL EXPERIMENTS—*continued*

B. After exposure to sunlight for 24 *hours*

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
71	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
72	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
73	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
74	+	—	+	+	+	—	—	—	+	III	*
75	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
76	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
77	+	—	+	+	+	—	—	—	+	III	*
78	—	—	+	+	+	—	—	—	+	III	*
79	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
80	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*

Group I, nil Group II, 1 Group III, 9 Group IV, nil

C. After exposure to sunlight for 48 *hours*

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
81	—	—	+	+	—	+	—	—	+	IV	*
82	—	—	+	+	—	+	—	—	+	IV	*
83	—	—	+	+	—	+	—	—	+	IV	*
84	—	—	+	+	—	—	—	—	+	IV	*B. coscoroba*
85	—	—	+	+	—	+	—	—	+	IV	*
86	—	—	+	+	—	+	—	—	+	IV	*
87	—	—	+	+	—	—	—	—	+	IV	*B. coscoroba*
88	—	—	+	+	—	+	—	—	+	IV	*
89	—	—	+	—	—	+	—	—	+	I	*
90	—	—	+	+	—	+	—	—	+	IV	*

Group I, 1 Group II, nil Group III, nil Group IV, 9

ADDITIONAL EXPERIMENTS—*continued*

D. After exposure to sunlight for 6 *days. Temperature of emulsion of fæces* = 37° C.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
91	—	—	+	+	—	+	+	—	+	IV	*
92	—	—	+	—	—	+	+	—	+	I	*
93	—	—	+	—	—	+	+	—	+	I	*
94	—	—	+	+	—	+	+	—	+	IV	*
95	—	—	+	+	—	+	+	—	+	IV	*
96	—	—	+	—	—	+	+	—	+	I	*
97	—	—	+	—	—	+	+	—	+	I	*
98	—	—	+	+	—	+	+	—	+	IV	*
99	—	—	+	+	—	+	+	—	+	IV	*
100	—	—	+	+	—	+	+	—	+	IV	*

Group I, 4 Group II, nil Group III, nil Group IV, 6

E. After exposure to sunlight for 10 *days. Temperature of emulsion* = 26° C.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
101	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
102	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
103	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
104	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
105	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
106	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
107	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
108	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
109	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
110	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*

Group I, nil Group II, 4 Group III, 6 Group IV, nil

* About all these there is a certain amount of doubt

ADDITIONAL EXPERIMENTS—*continued*

F. After exposure to sunlight for 30 *days. Temperature of fæcal emulsion* = 42° C.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
111	—	—	+	+	—	—	—	—	+	IV	*B. coscoroba*
112	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
113	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
114	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
115	—	—	+	—	—	—	—	—	+	I	*B. vesiculosus*
116	—	—	+	+	—	—	—	—	+	IV	*B. coscoroba*
117	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
118	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
119	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
120	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*

Group I, 1 Group II, 4 Group III, 3 Group IV, 2

To determine the types of lactose fermenters in soil heavily polluted by human excrement.

A. Samples taken from a trench 24 *hours old.* Date, December 17.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
201	+	—	+	—	—	—	—	—	+	I	*
202	+	—	+	+	+	—	—	—	+	III	*
203	+	—	+	—	+	—	—	—	+	II	*B. coli*
204	+	—	+	—	+	—	—	—	+	II	*B. coli*
205	+	—	+	+	—	—	—	—	+	IV	*
206	+	—	+	—	—	—	—	—	+	I	*
207	+	—	+	+	+	+	—	—	+	III	*
208	+	—	+	+	—	—	—	—	+	IV	*
209	+	—	+	—	+	—	—	—	+	II	*B. coli*
210	+	—	+	+	—	—	—	—	+	IV	*

Group I, 2 Group II, 3 Group III, 2 Group IV, 3

ADDITIONAL EXPERIMENTS—*continued*

B. Samples taken from a trench 7 *days old*

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
211	—	—	+	+	+	+	—	—	+	III	*
212	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
213	—	—	+	—	—	—	—	—	+	I	*B. vesiculosus*
214	—	—	+	—	—	—	—	—	+	I	*B. vesiculosus*
215	—	—	+	—	—	—	—	—	+	I	*B. vesiculosus*
216	—	—	+	+	+	+	—	—	+	III	*
217	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
218	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
219	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
220	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*

Group I, 3 Group II, 3 Group III, 4 Group IV, nil

C. Samples taken from a trench 14 *days old*

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
221	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
222	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
223	—	—	+	—	—	—	—	—	+	I	*B. vesiculosus*
224	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
225	—	—	+	+	+	+	—	—	+	III	*
226	—	—	+	+	—	+	—	—	+	IV	*
227	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
228	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
229	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
230	—	—	+	—	—	—	—	—	+	I	*B. vesiculosus*

Group I, 2 Group II, 3 Group III, 4 Group IV, 1

* About all these there is a certain amount of doubt

ADDITIONAL EXPERIMENTS—*continued*

To estimate the types of lactose fermenters present in the fæces of a goat.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
301	+	—	+	+	—	—	—	+	—	IV	*B. cloacæ*
302	+	—	+	—	+	—	—	—	+	II	*B. coli*
303	+	—	+	—	+	—	—	—	+	II	*B. coli*
304	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
305	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
306	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
307	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
308	+	—	+	—	+	—	—	—	+	II	*
309	+	—	+	—	+	—	—	—	+	II	*
310	+	—	+	—	+	—	—	—	+	II	*

Group I, nil Group II, 5 Group III, 4 Group IV, 1

Date, August 14, 1910

To estimate the types of lactose fermenters present in cow dung.

Colony number	Motility	Gram's stain	Lactose	Saccharose	Dulcit	Adonit	Inulin	Voges-Proskauer reaction	Indol	Group	Bacillus
311	—	—	+	+	—	+	—	—	+	IV	*
312	—	—	+	+	+	—	—	—	+	III	*B. neapolitanus*
313	—	—	+	—	+	—	—	—	+	II	*B. schæfferi*
314	+	—	+	+	+	—	—	+	+	III	*
315	—	—	+	+	+	+	—	+	+	III	*
316	—	—	+	+	+	—	—	+	+	III	*
317	—	—	+	+	+	+	—	+	+	III	*
318	—	—	+	+	+	—	—	+	+	III	*
319	+	—	+	+	+	—	—	+	+	III	*
320	+	—	+	+	+	—	—	+	+	III	*

Group I, nil Group II, 1 Group III, 8 Group IV, 1

List of Coli Group of Organisms of Fæcal Origin Isolated by Clemesha and his Assistants

Number		Lactose	Saccharose	Dulcit	Adonit	Inulin	P. & V. reaction	Indol	Motility	Gram's stain	Gelatine	Litmus milk	Acidity in litmus whey	Reduction of nitrates	Inosit
													Per cent.		
1		+	—	—	+	—	—	+	+	—	—	+	...	+	—
2	*B. acidi lactici* (Huppe)	+	—	—	+	—	—	+	—	—	—	+	23	+	—
3	*B. levans*	+	—	—	—	+	+	—	+	—	+	+	14	+	—
4	*B. grunthal*	—	—	—	—	—	—	+	+	—	—	+	20	+	—
	B. sulcatus gasiformans	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	B. castellus	...	...	...	...	...	...	...	...	...	...	...	...	...	...
5	*B. vesiculosus*	+	—	—	—	—	—	+	—	—	—	+	25	+	—
6		+	—	—	—	—	+	+	—	—	—	+	...	...	...
7		+	—	—	—	—	—	—	+	—	—	+	...	—	—
8	*B. coli mutabilis* (Massini)	+	—	—	—	—	...	—	—	—	—	+	...	...	...
9		+	—	—	+	—	+	+	—	...	...	...	...	...	...
10		+	—	—	—	+	—	...	...	...	...	...	...	...	...
33		+	—	+	+	—	—	+	+	+	—	—	...	...	—
34	*B. coli communis*	+	—	+	—	—	—	+	+	—	—	+	28	+	—
	B. cavicidæ	+	—	...	...	...	...	...	...	...	...	...	...	...	...
35	*B. schæfferi*	+	—	+	—	—	—	+	—	—	—	+	22	+	—
36		+	—	+	—	—	—	—	+	—	+	+	...	+	—
37		+	—	+	—	—	+	...	—	...	...	...	...	...	...
38		+	—	+	+	—	+	...	...	...	...	...	...	...	...
39		+	—	+	—	+	—	...	—	...	...	...	...	...	...
65	*B. oxytocus perniciosus*	+	+	+	+	+	+	+	—	—	+	+	26	+	+
66		+	+	+	+	—	—	+	—	—	—	+	...	+	—
67		+	+	+	+	—	+	—	—	—	—	+	...	+	+
68	*B. rhinoscleroma pneumoniæ*	+	+	+	+	—	—	—	—	—	—	+	14	+	+
69		+	+	+	—	+	+	—	+	—	+	+	...	...	—
70		+	+	+	—	+	—	—	+	—	+	+	...	...	—
71		+	+	+	—	—	—	+	+	—	—	+	...	+	—
72	*B. neapolitanus*	+	+	+	—	—	—	+	—	—	—	+	22	+	—
73		+	+	+	—	—	+	—	+	—	+	+	...	+	—
74		+	+	+	—	—	—	—	+	—	—	+	...	...	...
75		+	+	+	—	—	+	—	—	—	—	+	...	...	+
97		+	+	—	+	+	+	+	—	—	+	+	...	+	—

Continued on next page

List of Coli Group of Organisms of Fæcal Origin Isolated by Clemeshâ and his Assistants—*continued*

Number		Lactose	Saccharose	Dulcit	Adonit	Inulin	P. & V. reaction	Indol	Motility	Gram's stain	Gelatine	Litmus milk	Acidity in litmus whey	Reduction of nitrates	Inosit
													Per cent.		
99		+	+	—	+	+	—	—	—	—	—	+	...	+	+
100		+	+	—	+	—	—	+	—	—	—	+	...	...	...
101		+	+	—	+	—	—	+	—	—	—	+	...	+	...
102		+	+	—	+	—	+	—	+	—	+	+	...	...	...
103	*B. lactis ærogenes*	+	+	—	+	—	+	—	—	—	—	+	30	+	+
	B. dysentericæ vitulorum	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	B. capsulatus (Pfeiffer)	...	...	...	...	...	...	...	...	...	...	...	...	...	...
104	*B. gasiformans non-liquefaciens* ...	+	+	—	+	—	—	—	—	—	—	+	12	+	—
105		+	+	—	—	+	+	—	+	—	+	+	...	...	—
106		+	+	—	—	—	—	+	+	—	—	+	...	...	—
107	*B. coscoroba*	+	+	—	—	—	—	+	—	—	—	+	25	+	—
108	*B. cloacæ*	+	+	—	—	—	+	—	+	—	+	+	20	+	—
109		+	+	—	—	—	—	—	+	—	—	+	...	...	—
110		+	+	—	—	+	—	...	...	...	...	...	...	...	...
98		+	+	—	+	+	+	—	—	—	—	+	...	+	+

Some of the *coli* group of organisms of fæcal origin classified by Clemesha according to their powers of resisting sunlight.

Class I. = Those feebly resistant.

Class II. = Intermediate.

Class III. = Very resistant.

CLASS I	CLASS II	CLASS III
**Oxytocus perniciosus*	No. 33	No. 73
*No. 10	No. 38	No. 75
*No. 39	*Rhinoscleroma pneumoniæ*	*Grunthal* or No. 7 or 8
*No. 69	No. 9	*Cloacæ*
*No. 70	*Neapolitanus*	
*Nos. 97 and 98	*Lactis ærogenes*	
Coli communis	*Coscoroba*	
No. 35	*Acidi lactici*	
No. 36	No. 6	
	No. 100	
	No. 101	

* About all these there is a certain amount of doubt

Experiments on the Filtering Properties of the Zeer

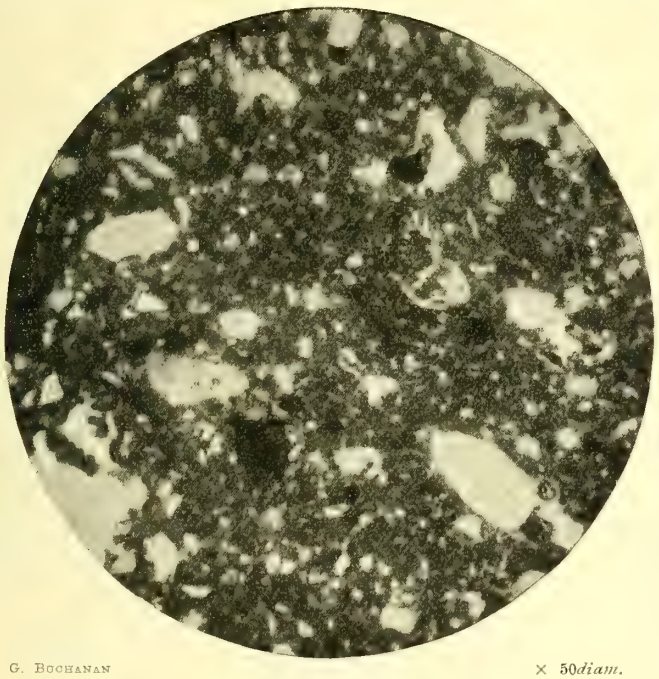
by

Captain R. G. Archibald, M.B., R.A.M.C., attached E.A.

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The zeer is one of the most useful domestic utensils employed in Egypt and the Sudan. In these two countries its use is practically universal, for not only is it serviceable as a receptacle for holding water, but also as a filtering medium for removing any turbidity.



G. Buchanan × 50*diam.*

Fig. 102.—Section of a Zeer

Up till a short time ago, and before Khartoum was provided with a water-supply, it was used by all classes to clear the drinking-water taken from the river, and, while these conditions existed, a few rough experiments were carried out to test its filtering capacity.

Several types of zeers are manufactured by the natives, and the commonest or white variety is composed of clay moulded into a suitable shape and then baked at a high temperature. If a section of a zeer be made and examined microscopically, it will be seen to consist of a somewhat coarse reticulum of clay containing portions of quartz and hæmatite (*vide* Fig. 102).

In carrying out these experiments with the zeer, the procedure adopted was to fill it with 10 litres of river water and to estimate the number of colonies of organisms present. Samples of varying amounts of water were taken and plated out on agar. Samples were then taken from the filtrate, and the colony count per 1 c.c. estimated in a similar manner.

Experiment I. New unused zeer.

Average colony count per 1 c.c. water in the zeer = 340.

Average colony count per 1 c.c. of immediate filtrate = 236.

After the water had been in the zeer for 24 hours, the latter was emptied, and a fine slimy deposit was found adherent to its inner surface. This deposit was not disturbed. In order to see whether this deposit acted at this stage in any way as a vital layer, ten litres of water were poured into the zeer, and samples tested after 24 hours with the following results:—

Action of the slimy deposit

Average colony count of water in zeer = 200 odd.

Average colony count of filtrate = 400 odd.

This experiment was repeated several times, and it was found that the colony count of the filtrate always exceeded that of the water contained in the zeer. One was led, therefore, to conclude that at first this slimy deposit acted as a culture medium in which the water organisms multiplied.

The inner surface of the zeer was then scrubbed out with a brush, the vessel was refilled, and the water allowed to remain in it for 14 days. The results are tabulated:—

Experiment	Time after zeer had been cleaned	Colony count per 1 c.c. of filtrate	Count per 1 c.c. of river water in zeer
1	Twenty-four hours	350 odd	These averaged as a rule between 300 and 400 per 1 c.c.
2	Two days	115	
3	Three days	17	
4	Four days	6	
5	Five days	12	
6	Six days	10	
7	Seven days	14	
8	Eight days	31	
9	Nine days	58	
10	Ten days	52	
11	Twelve days	128	
12	Fourteen days	about 300 odd	

Optimum conditions for Zeer-filtration

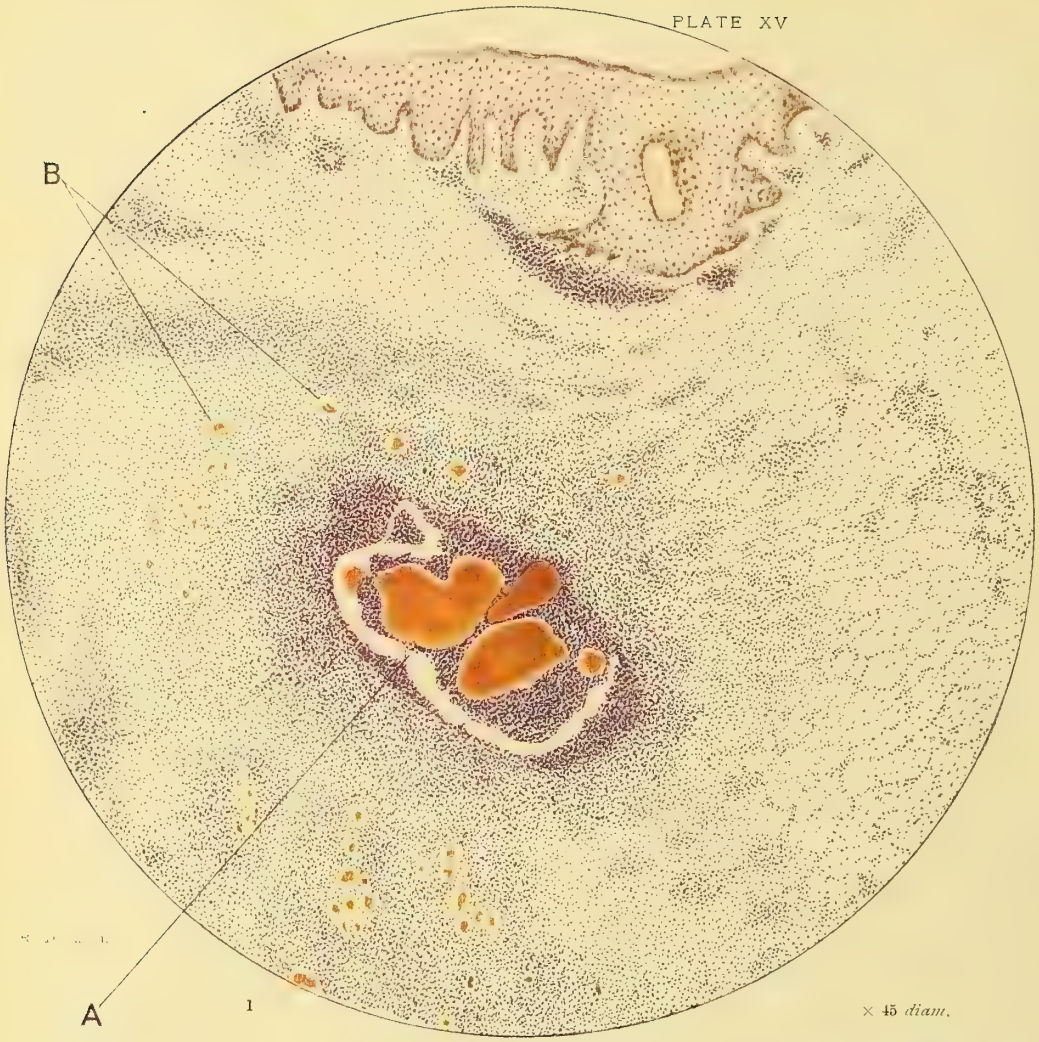
The results obtained showed that, in order to get the best filtering properties out of a zeer, it is necessary to allow water to stand in it for a period of four days till a considerable slimy deposit is formed on its inner surface, presumably also extending into its pores. The lowest colony counts in the filtrate occurred between the second and ninth days, an indication, therefore, that the zeer ought to be cleaned out every ten days, as thereafter organisms grow through the vital layer and appear in numbers in the filtrate.

A chemical analysis of the water in the zeer and in the filtrate was kindly carried out by Mr. J. A. Goodson, F.I.C., Assistant Chemist, with the following results:—

Water in the Zeer		Filtrate	
Total solids	998·0	138·4	parts per million
Solids in suspension	67·63	4·8	,, ,, ,,
Dissolved solids	130·37	133·60	,, ,, ,,
Free ammonia	·060	·101	,, ,, ,,
Albuminoid ammonia	·579	·302	,, ,, ,,
Oxygen consumed	16·44	3·66	,, ,, ,,
Nitrates	·150	·181	,, ,, ,,
Nitrites	Nil	trace	,, ,, ,,
Chlorides	1·9	3·42	,, ,, ,,
Sulphates	1·03	2·88	,, ,, ,,
Carbonates...	42·37	47·78	,, ,, ,,

The increase in carbonates, sulphates, and chlorides was doubtless caused by the presence of these salts in the zeer material.

PLATE XV



1

1. Botryomycosis

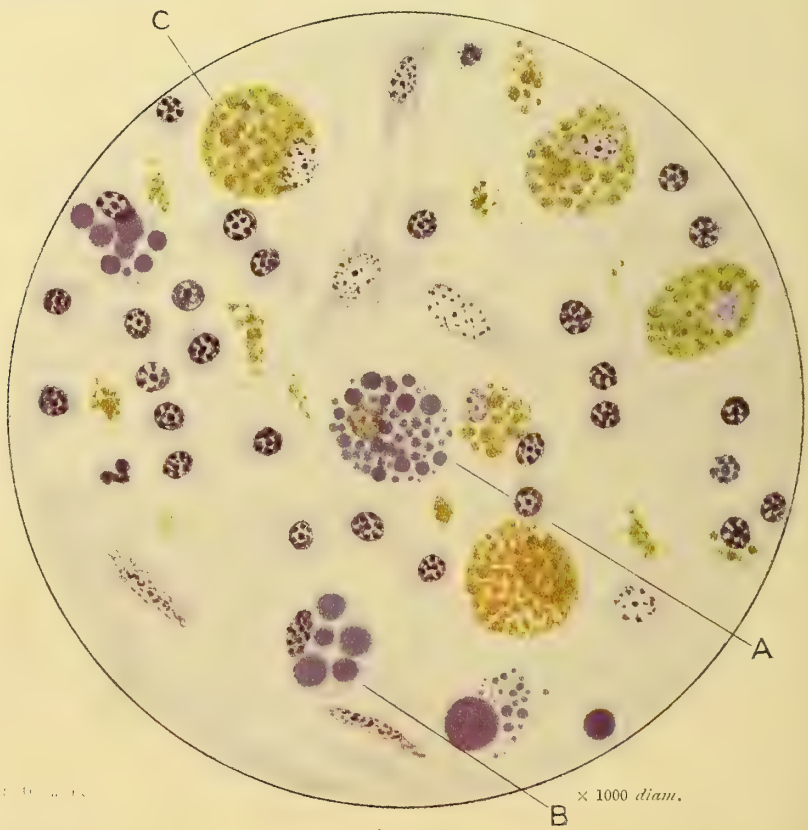
Showing

A round-celled nodule of a lymphoid type lying in the subcutaneous tissues

B pigment

Hæmatoxylin and Eosin Stain

× 45 *diam.*



2

2. Botryomycosis

Showing

A and B, coccus bodies of varying sizes ingested by phagocytic cells

C phagocyted pigment

Gram-Weigert Stain

× 1000 *diam.*

*Human Botryomycosis

BY

Captain R. G. Archibald, M.B., R.A.M.C., attached E.A.

Pathologist and Assistant Bacteriologist

Wellcome Tropical Research Laboratories, Khartoum

Within the past year opportunities occurred of studying a peculiar pathological condition in certain tumours which had been sent to the Wellcome Tropical Research Laboratories from various parts of the Sudan. The first of these was a tumour removed from the scalp of a native. Stained sections made from this specimen showed the presence of agglomerations or collections of circular disc-like masses lying irregularly throughout the tumour mass. When stained by the aniline dyes they exhibited a homogeneous appearance without any apparent intrinsic structure, and closely resembled collections of staphylococci of varying sizes. Similar appearances had been previously observed by Dr. Balfour in a growth removed from a camel, and had been recognised by him to be botryomycotic in nature.

After reference to all the available literature had been made, the conclusion arrived at was that these collections of coccus-like masses also represented a pathological entity met with in a somewhat rare disease named botryomycosis, and, in fact, were pathognomonic of it, the disease itself deriving the name of botryomycosis from the resemblance these collections of coccal masses bear to a cluster of grapes. Since this first case no less than seven other tumours, removed from the breast, arm, hand, foot and scrotum, have been found to contain similar coccus-like agglomerations. Three of these tumours had, in addition, all the pathological macroscopical appearances associated with *Streptothrix maduræ* and its presence was subsequently confirmed microscopically. In the others there was no evidence of a streptothrix existing, although a careful search was made through a large number of sections taken from various parts of the growths.

Association with *Streptothrix* in some cases

It may be stated that one was somewhat diffident in naming the condition botryomycosis but for the fact that the pathology of these growths closely simulated the description given of this disease by Ernst[1] and other observers, who found these collections of coccus-like masses present in certain lesions in animals, notably the horse, cow and hog, and who, for some time, described them as staphylococcal in character. This staphylococcal view, however, is no longer tenable, for special staining reactions have shown them to be of a different nature. Cultural and inoculation experiments would doubtless assist in clearing up the exact nature of these bodies, but, unfortunately, opportunities of examining fresh pathological material have not occurred, and, as a result, the work embodied in this paper is somewhat incomplete. Kitt,[2] in his text-book on comparative pathology, defines botryomycosis as a productive inflammation leading to the formation of fibrous growths attended invariably with suppuration and fistula formation, or the production of yellowish-red foci of softening caused by infection with the *Botryomyces ascoformans*.

* Reprinted from the *British Medical Journal*, October 1, 1910, being a paper read before the British Medical Association, 1910.

[1] Ernst, W. (1908), "Die Enstehung der Botryomycosrasen aus der Staphylokken form des Erregers." *Centralbl. f. Bakt.*, 1 Abt. Originale, Vol. XLV., No. 2.

[2] Kitt, Th. (1906), "Botryomycosis." *Comparative General Pathology.*

As already mentioned, its pathological effects in animals have been studied by a few Continental observers. The horse, cow and hog appear to be more susceptible to the disease than other animals. More recently its occurrence in man has also been noted by Bureau and Labbé. Bitter of Cairo has found it in Egyptian cases.

The Clinical Signs of the Disease

Males seem to be more commonly affected than females. One case occurred in a native woman aged 45, who had suffered from a swelling in her breast since childhood. For the past two years, however, the condition had occasioned her annoyance, and, on presenting herself for treatment, the breast was found to be considerably inflamed, exhibiting sinuses which exuded a greyish-white pus. The whole of the breast tissue was involved, the growth simulating a fungating carcinoma, but the lymphatic glands in the axilla and neck were not enlarged. The breast was excised by Dr. Hodson, of the Sudan Medical Department, who kindly sent the specimen.

Tendency to a chronic course

From the histories obtained in the other cases, the disease appears to be more or less a chronic one, commencing, as a rule, in the form of a superficial nodule, which increases in size, and invades the deeper tissues, eventually involving fascia, muscle, tendon, and sometimes bone. Specimens illustrating this are seen in the appended photographs of the disease affecting the hand and foot where the resemblance to mycetoma is a somewhat striking one.

The tumour of the scalp kindly sent by Captain Thomson, R.A.M.C., involved the left occipito-temporoparietal region. The history of this case is of interest.

The growth commenced about twelve months previously as a small lump about the size of a date, and remained unchanged in dimensions till the patient was advised to have a red hot iron passed into the tumour to kill it. This drastic treatment was carried out, with the result that might be expected, namely, a marked increase in size. The tumour was movable, but appeared to be adherent in parts to the subjacent bone. When the growth was excised the subjacent bone was found to be soft and slightly infiltrated. The sinuses on the surface exuded a thin watery pus containing yellow-white granules.

The surface of this tumour was covered with sinuses around which were heaped up masses of white fibrous tissue. This proliferating fibrous tissue was also present around the openings of the sinuses in all the other specimens. The sinuses extended to varying depths, and were lined by granulation and necrosed tissue. In the hand and foot specimens, they extended down to tendon and bone, leading to considerable contracture and deformity. If a section be made through one of these growths its fibrotic nature will be at once apparent, the intervening tissue between the sinuses consisting, for the most part, of white fibrous tissue. This is frequently arranged in whorls, sharply demarcating yellow hyaline-looking areas, which microscopically are seen to be composed of groups of fat cells. Further, lying in this fibrous tissue, masses of yellow-brown pigment are visible to the naked eye.

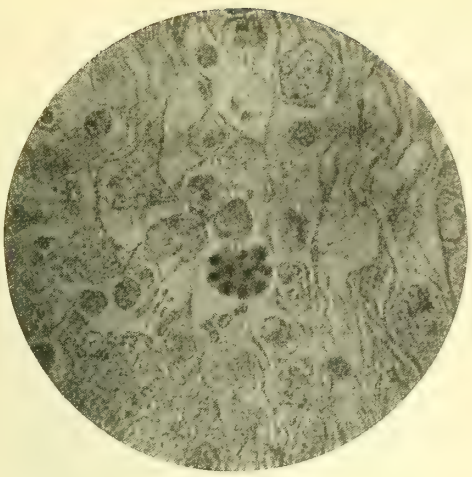
Fibrotic changes

The microscopical pathological changes are very constant, and consist of:—

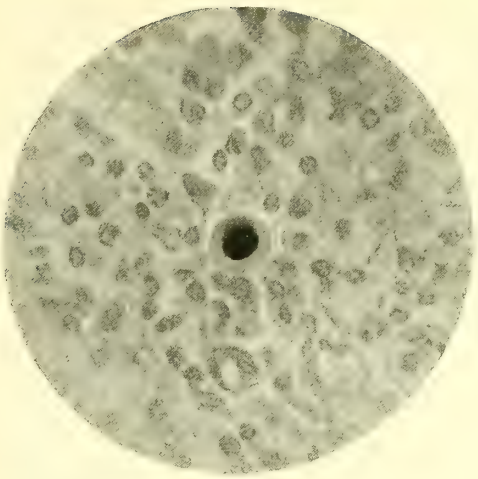
1. Marked proliferation of fibrous tissue which, in certain parts of the section, appear to be undergoing myxomatous degeneration, more especially in the areas surrounding the sinuses. Lying in this fibrous tissue are reticulated areas, which contain fat globules.

2. Round cell proliferation of a lymphoid type, suggesting the appearance of a tubercle nodule (Plate XV., fig. 1). Giant cells are occasionally met with.

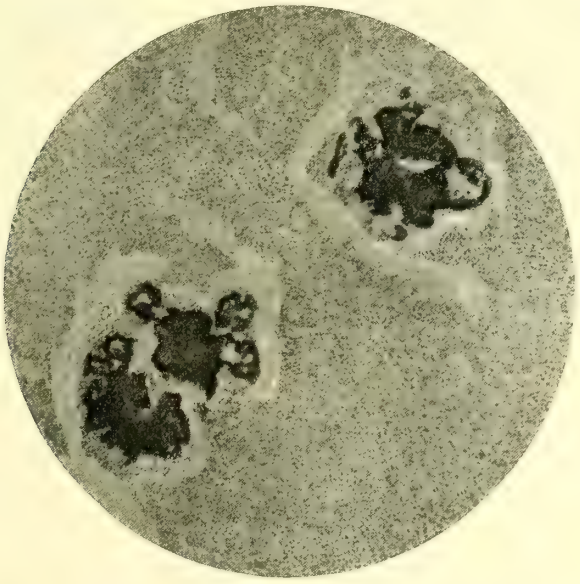
PLATE XVI



1



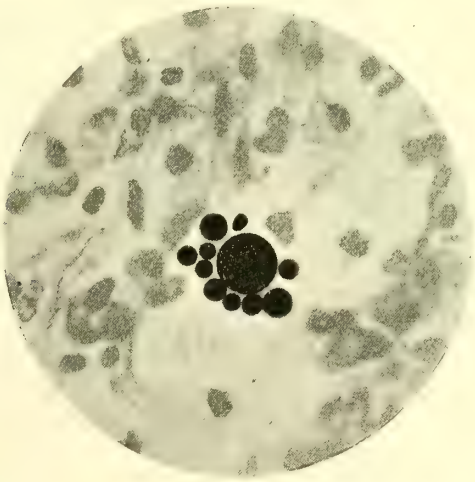
2



3



4



5

G. Buchanan

BOTRYOMYCOSIS

1. Botryomycogenous cell.
2. Showing the protoplasmic ring around a "coccus."
3. Botryomycotic areas of a mycetomatous type.
4. Showing clumps of "cocci."
5. Showing large clumps of coccal forms.

Nos. 1 and 2. *Iron Hæmatoxylin and Eosin Stain*
Nos. 3–5. *Gram-Weigert Stain*

No. 3 × 80 *diam.*
Nos. 1, 2, 4 and 5 × 600 *diam.*

3. Changes in the blood-vessels, there being a periarteritis and a proliferation of the tunica intima and tunica media.

4. Presence of pigment scattered irregularly throughout the tissue in the form of yellow-brown granules lying free, and also phagocyted. Special staining methods showed this to be an iron-containing pigment (Plate XV., fig. 2, C).

Microscopic appearances

5. The presence of large cells of an epithelioid type lying in necrosed tissue and infiltrating the surrounding fibrous tissue. These epithelioid cells are, for the most part, oval in shape, and contain a nucleus and nucleolus, and some show vacuoles in their cytoplasm. They measure 0·8μ to 12·8μ, and appear to have considerable phagocytic properties, for many of them contain ingested pigment.

6. Groups of botryomyces lying irregularly scattered throughout the section. These, as already stated, resemble large staphylococci, and occur sometimes singly or more frequently in clumps of coccal masses three to twelve in number. They vary in size from 0·8μ to 12·8μ. On focussing one of these clumps, one finds that all the coccal masses do not focus in the same plane, some appearing more superficial than others (Plate XVI., figs. 4 and 5).

Staining reactions

By the ordinary staining methods these coccus-like masses show no differentiated structure. They retain Gram's stain. Owing to their structureless appearance and the fact that their presence in various parts of the section was not associated with any surrounding inflammatory changes, the possibility of their being amyloid or glycogenic in nature indicated that special staining reactions should be employed. The results of these, however, threw no light on their chemical composition for they gave neither the amyloid nor the glycogenic reaction.

All were acid-fast, and by using special stains a zooglœa-like mass could be seen holding together, as it were, these coccal-like bodies.

Several sections had been examined before any idea was obtained as to the probable method of origin of these coccal masses.

When Muir's method of capsule staining is employed, they take up the fuchsin stain, the methylene blue staining what is evidently a thin rim of protoplasm around each coccus (Plate XVI., fig. 2). Parts of the section, however, when stained by Muir's method, also show two, three, and four coccal discs lying in different focal planes, and clumped together within what appears to be a cell envelope, the whole representing what one may assume to be the botryomycogenous cell (Plate XVI., fig. 1) described by the French observers. These botryomyces, when intra-cellular, appear to multiply within the cell envelope till the latter eventually ruptures allowing escape of the individual cocci, which in turn appear to be taken up by the blood and lymph stream and carried to various parts of the tissues. Sections made from the growth in the scrotum showed these individual coccal masses occupying the lumen of blood-vessels. This method of transmission by means of blood and lymph vessels possibly explains the presence of some of these botryomyces in apparently healthy fibrous tissue, their presence there, as already stated, not being associated with any inflammatory changes. While, however, these botryomyces are intra-cellular, they apparently bring about degenerative changes in the surrounding tissue which is more or less necrosed. After the cell envelope has ruptured, these coccal masses evidently undergo some form of degeneration of a hyaline type and assume different staining reactions, for, in sections stained by hæmatein and eosin, the intra-cellular coccal masses take up the nuclear stain of the hæmatein, while the escaped extra-cellular ones show an affinity for eosin and appear

Degeneration changes



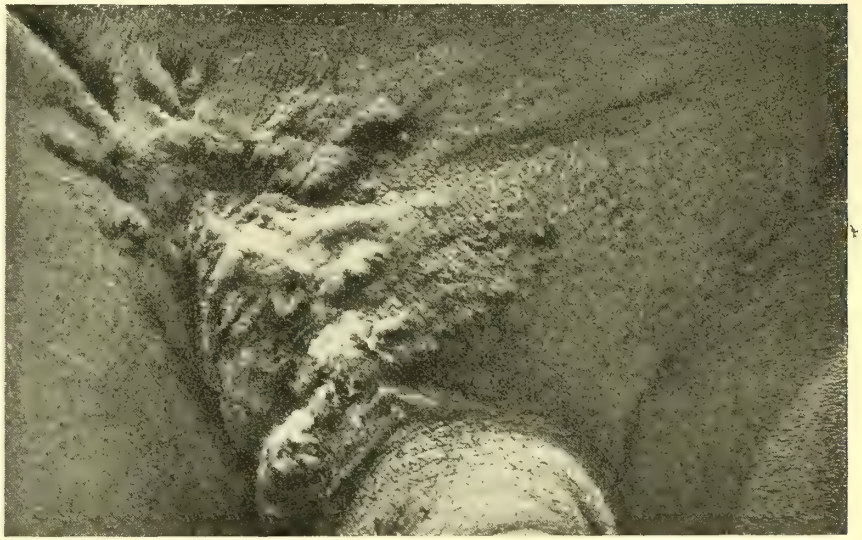
G. Buchanan

Fig. 103.—Botryomycosis of the Upper Jaw



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Fig. 104.—Botryomycosis of Portion of Hand



G. Buchanan

Fig. 105.—Botryomycosis of the Groin

more hyaline in consistence. Even when enclosed within the cell and prior to rupture of the cell envelope, these coccal masses sometimes undergo degenerative changes, taking on almost a neutral staining tint, showing neither a distinct affinity for the hæmatein nor for the eosin.

Similar staining reactions were obtained with Leishman's stain, the coccal masses taking up the nuclear stain when occupying a position within the cell envelope, and showing an affinity for the eosin when extra-cellular, appearing at the same time more hyaline-looking and structureless in contrast to the granular and often vacuolated appearance exhibited when occupying their intra-cellular position. Sections stained with iron hæmatoxylin alum and counter-stained with saffranin show very well the central nuclear structure of these individual botryomyces surrounded by a saffranin staining rim of cytoplasm, which, in some cases, appears striated.

Further work requires to be done to establish the exact nature of these botryomyces, and their possible mode of transmission to man. As already stated, considerable assistance would be obtained if one had access to fresh pathological material, but so far opportunities for this have been lacking. In animals the disease is transmitted by direct infection through a wound or abrasion of the skin, especially in those parts of the skin subjected to friction. Man, in all probability, becomes infected in a similar manner, but the possible rôle played by ticks and other biting insects would have to be considered.

Conclusions

Possible connection with a streptothricosis

From the fact that a streptothrix condition was present in three of the tumours sent for examination, one is inclined to come to the conclusion that botryomycosis is really a streptothricosis and that the clumps of botryomyces in reality represent the gonidia of possibly a new and hitherto undescribed species of streptothrix.

Reference has been made to all the literature available here including Brumpt's[1] excellent monograph entitled "Les mycetomes" but one has failed to find any work giving a similar description of these botryomyces.

Further, the clinical appearances of three of the tumours certainly support the streptothrix theory of this disease. Before concluding, reference may be made to the amœbic theory of the disease put forward by Bureau and Labbé.[2] These observers found in a case of botryomycosis of the finger, a large amœba measuring 50μ to 60μ thus apparently confirming Letulle's[3] previous view that possibly botryomycosis is amœbic in origin. Several staining methods have been employed with the view of confirming, if possible, Labbé and Bureau's amœbic theory, but so far without success. Certainly it is difficult to associate such a large amount of destruction of dense tissue as is met with in botryomycosis with the presence of an amœba.

[1] Brumpt, E. (November 25, 1906), "Les mycetomes." *Arch. de Parasit.*, Vol. X., No. 4.

[2] Bureau, G., and Labbé, A. (July 15, 1909), "Sur l'affection connue sous le nom de botryomycose et son parasite." *Bulletin de l'Institut Pasteur.*

[3] Letulle, M. (July 15, 1908), "La botryomycose." *Bulletin de l'Institut Pasteur.*

Veterinary Notes

by

The Director

In view of the immense importance of scientific veterinary work in the Sudan, I think it advisable to submit a few observations regarding diseases of animals. These are, of course, entirely from the laboratory standpoint and it is pleasant to record the great assistance, both with notes and specimens, which the officers of the Sudan Veterinary Department have always accorded the laboratories. Some day, I trust we may see one of them on the laboratory Staff, engaged wholly in investigations which are bound to be fruitful both from the scientific and economic aspect.

Epizootic Lymphangitis of Equines

Plate XVII., fig. 5 is an illustration of the parasite of epizootic lymphangitis, a common disease of equines in the Sudan, and one at times occasioning heavy losses to Government.

All kinds of ideas have been advanced regarding its nature since it was claimed as a cryptococcus and named *Saccharomyces farciminosus* by Rivolta.

Recent investigations

Of recent years it has been the subject of many investigations. In 1908, Pricolo[1] reviewed the whole subject, pointing out that the majority of observers regarded it as a blastomycete, but that Gasperini had sought to claim it as a protozoon. He also cited the experiments of Sanfelice, Tokishige, Marcone and Baruchello, which all go to prove that it is of a mycotic (fungoid) nature. Sanfelice cultivated the organism and observed the formation of filaments. Piana and Galli-Valerio thought it was a sporozoon and Gasperini,[2] following Canalis, believed it to be a coccidium and proposed for it the new name, *Lymphosporidium equi*.

Mori,[3] who, like Gasperini, failed to cultivate it, or to produce lymphangitis in experimental animals by its inoculation, also believed it to be a protozoon. Ducloux, of Tunis, found in the mule a form which he also regarded as protozoal, and, after some study, concluded it to belong to the group Phagocytozoa and named it *Leucocytozoon piroplasmoides*. Theiler[4] finds that in the Transvaal the lymphangitis of equines is due to *Saccharomyces farciminosus*.

Thiroux and Teppaz[5] agree with Ducloux, and point out the close resemblance of the parasite to *Leishmania tropica*. The more recent work of Galli-Valerio[6] led him to adopt the protozoal theory, to support Thiroux and Teppaz, and to name the organism *Leishmania farciminosa*.

[1] Pricolo, A. (1908), "Contribution to the Study of Epizootic Lymphangitis." *Revue Gén. de Méd. Vét.* quoted in *Journal Tropical Veterinary Science*, Vol. III., p. 217.

[2] Gasperini, G. (May 14, 1908), "La linfangite protozoaria ed il suo agente specifico Lymphosporidium equi." *Acc. Med. fis. Fiorent*, p. 31.

[3] Mori, N. (1908), "Osservazioni sul considetto farcino criptococcico linfangite epizootica o saccaromicosi equina." *La clinica veterin.*

[4] Theiler, A. (1908), "Tierseuchenbekämpfung in Transvaal." *Deutsche tierärztl. Woche*, Vol. XIV.

[5] Thiroux, H., and Teppaz, L. (1908), "Sur le Leucocytozoon piroplasmoides (Ducloux) de la Lymphangitis Épizootique des Équidés." *C. R. Acad. de Sciences*, pp. 1075–1076.

[6] Galli-Valerio, B. (1909), "L'état actuel de nos connaissances sur l'agent specifique de la lymphangite épizootique des Équidés." *Centr. f. Bakt. 1 Abt. Ref.*, Bd. 44 pp. 577–582.

Panisset,[1] while not committing himself to an opinion, thought that the arguments advanced in favour of the protozoal view were not adequate, while Bridé and Nègre[2] have applied the complement deviation test, and as a result conclude that the parasite is undoubtedly a yeast. It is possible that all this confusion is due to more than one kind of parasite being described, but, so far as the Sudan goes, there is every probability that the organism is mycotic. Bitter has cultivated it in Egypt, and finds that its growth is quite characteristic and it is not likely that the Sudan disease differs from the Egyptian. There has been no necessity to carry out research work on the subject here, but this brief review and the accompanying illustration may prove helpful (Plate XVII., fig. 5).

Coccidiosis in Cattle

Montgomery[3] drew attention to the presence of Coccidiosis in cattle in British East Africa, described the symptoms, and warned observers in Uganda and the Sudan to be on the look-out for this disease which was spreading north from German East Africa.

Dr. A. C. Stevenson, when working in these laboratories during the winter 1907–8, discovered a coccidiosis in goats which he describes in this Report (*vide page* 355).

Recently I visited Wau in the Bahr-El-Ghazal and there examined two herds of sick cattle from the Atwot country in the south. I found one herd suffering from piroplasmosis (infection with *P. bigeminum*), but the cattle examined in the other herd showed no parasites and appeared from their history to be recovering from rinderpest. I noticed they presented a scaly condition of the skin and marked anæmia, while some still exhibited nasal discharge. At Kodok on the White Nile I was asked to see some sick cattle, and examined the blood of an ox dying of what certainly appeared to be rinderpest. It presented all the classical symptoms. The blood examined was negative, and I had to leave before the animal died so that no post mortem was made. On revisiting Kodok I found a small herd of sick cattle which were weak, anæmic, and appeared to have a mild attack of rinderpest. Some of the herd had died. I examined the blood of one of these animals, with negative results. Now it so happened that two goats had accompanied me on my journey. They had been put ashore for grazing purposes at several places on the White Nile, and had remained behind at Meshra-El-Rek while one journeyed overland to Wau. A few days out from Khartoum, on the return voyage, one of the goats was noticed to be ill. Its most marked symptom was an affection of the hide, the skin becoming scurfy and the hair loose. Dr. Stevenson had noticed this in the goats he examined. It had lost appetite, and was getting emaciated. On reaching Khartoum, it was chloroformed and examined, as I suspected it was a victim of coccidiosis. This proved to be the case. On killing the companion goat, which was apparently healthy, a similar infection was found to be present. In both cases the small intestine was the portion of gut infected.

Goats found infected

In the light of these facts, therefore, and the timely warning issued by Montgomery, it appears to me that the cattle both at Wau and at Kodok, which appeared to have rinderpest, may very well have been suffering from coccidiosis. I have drawn the attention of the Civil Secretary of the Sudan Government to the matter, and have suggested that a Veterinary Officer be sent to investigate the disease fully, and if it is found to be coccidiosis,

[1] Panisset, L. (April, 1909), "La Place zoologique du Parasite de la Lymphangite Épizootique." *Rev. Gen. Méd. Vét.*, pp. 378–384.

[2] Bridé, J., and Nègre, L., "Sur la Nature du Parasite de la Lymphangite Épizootique." *C. R. Acad. Sciences*, pp. 998 and 1265.

[3] Montgomery, E., *Bulletin de la Societé de Pathologie Exotique*, Tome III., May 11, 1910, No. 5, pp. 293–296.

to take such precautions as may be necessary. I am also endeavouring to obtain material for examination, and only regret I did not see Montgomery's suggestive paper before I travelled south.

Piroplasmosis

(*a*) *Canine.* This exists to a considerable extent in Khartoum, and the carriers of the parasite are *Rhipicephalus sanguineus* and *Hæmaphysalis leachi.* There is nothing special to note about the disease. In severe attacks the blood shows great anæmia and much alteration in the red cells. Captains Fry and Archibald have employed trypan blue in several cases with success, and the discovery of Nuttall and Graham-Smith[1] will prove of great service, as hitherto it has scarcely been possible to keep any but native dogs as pets. These latter take the disease and sometimes die from it, but I expect that, for the most part, they suffer as puppies and so acquire a certain immunity.

(*b*) *Equine.* As stated previously, this occurs to some extent in the Sudan. Whether the idea that it was originally imported from South Africa is correct I cannot say, but I think it is more likely to be indigenous. I have studied the condition to some extent in donkeys, and Plate XVII., fig. 4, shows the type of parasite found. The animals came from Malakal and the ticks found on them were *Rhipicephalus evertsi.* Three sick donkeys were sent. Two had probably suffered from the disease and were convalescent, the other showed severe infection and typical petechial spots on the conjunctivæ. There was no red water but the urine was heavily laden with urates.

The parasite appears to be the typical *Nuttallia equi.* Spherical, pyriform, bacillary, irregular and rosette forms were all present.

Experimental work on transmission with the ticks found on the donkey was carried out by Dr. Aders, but gave only negative results. Tick experiments negative

The animal died and the post mortem examination revealed nothing of note. The spleen was carefully examined to see if any bodies resembling those described by Koch in East Coast Fever could be found, but only piroplasmata were present. (See, however, note on Anaplasmosis.)

(*c*) *Bovine.* Since the date of the last Report no further cases of the disease in cattle have come under notice at Khartoum, so it has not been possible to carry out any investigations regarding it. I found *P. bigeminum* in cattle at Wau in the Bahr-El-Ghazal in May, 1910.

* Anaplasmosis

As is now well known, Theiler[2] has declared his "marginal points" to be *Anaplasma marginale,* a specific protozoal parasite of cattle, and has apparently proved it to be the cause of gall-sickness in South Africa. Spreull[3] has also declared his conviction that the "marginal points" are parasitic in nature. If we accept these views it follows that gall-sickness exists in cattle in the Sudan, for I have found *Anaplasma marginale* in the blood of cattle suffering from piroplasmosis (*vide* Third Report, Plate IV., fig. 4). It was with some surprise, therefore, that after reading Theiler's article I remembered having noted that *Anaplasma marginale* was present in large numbers in the blood of the donkey from

[1] Nuttall, G. H. F., and Graham-Smith, G. S. (1909), "Notes on Immunity in Canine Piroplasmosis." *Parasitology,* Vol. II., pp. 215-228.

[2] Theiler, A. (June, 1910), "Gall Sickness of South Africa (Anaplasmosis of Cattle)." *Journal of Comparative Pathology and Therapeutics.* Vol. XXIII., Part 2.

[3] Spreull, J. (December, 1909), "Marginal Points, or a new Intra-corpuscular Parasite in the blood of Cattle in South Africa." *Ibid.,* Vol. XXII., No. 4.

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Malakal suffering from piroplasmosis. I confirmed the observation on re-examining the slide and noted with interest that Theiler states that hitherto anaplasmosis has only been observed in cattle. Some notes, therefore, on anaplasmosis of the donkey may not be out of place.

Anaplasmosis in a donkey

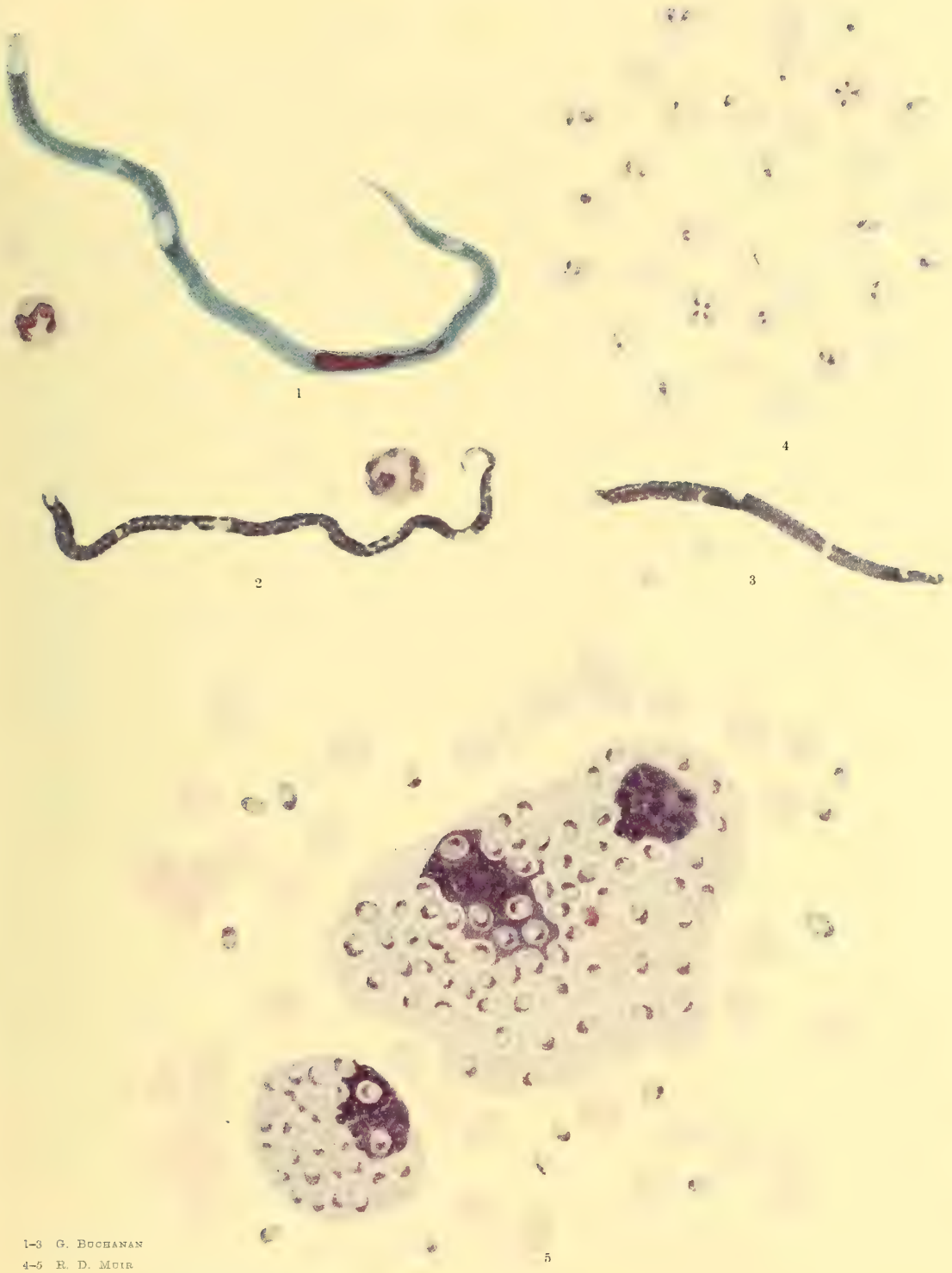
In the first instance the history of the case may be stated. The animal was one of three donkeys sent me from Malakal, on the White Nile, in November, 1908. On arrival, two of these appeared to be convalescing but showed "marginal points" in their bloods; the third, that under consideration, was infected with *Nuttallia equi* and also showed numerous "marginal points." These donkeys came from a herd belonging to the Irrigation Department which, during a period of two months, was reduced in numbers by sickness from 90 to 63. I could not obtain any definite account of the symptoms. The animals were said to have suffered much from skin lesions caused by picketing ropes and from the bites of flies (probably *Stomoxys*). There was loss of appetite, and towards the end, great weakness. Here, unfortunately, I only paid special attention to the donkey with piroplasmosis and its symptoms were those of that disease. Plate XVII., fig. 4, which is a composite drawing, shows very well the different forms of "marginal points" encountered, and it will be seen that they are very similar to those met with in cattle. Thus we have large and small spherical dots or coccus-like bodies consisting wholly of chromatin substance. Some tend to be slightly oval, and in certain cases a polar zone, where the staining is feeble, is evident. Double forms are not uncommon and seem to show, as Theiler says, that the bodies increase by fission.

The situation of the bodies in their host cells varies. Some are truly marginal, but the majority are a short distance from the edge, and quite a number are central. Indeed, if there is any difference between this *Anaplasma* and that of cattle, it is that the former is not so frequently marginal as the latter, and also that it attains to a size which the other does not. Some are, comparatively speaking, very large, and it is noteworthy that these large forms, which may be those prior to division, are often found in megalocytes. On the other hand, I have seen what I take to be exceedingly minute forms—only to be distinguished from tiny specks of dirt by their definite outline and chromatin red colour. Are those the youngest forms? I have seen a large and a very small form far apart in the same red cell, but multiple infection is distinctly rare. A body may occupy the same corpuscle with *Nuttallia equi*, but this is uncommon. As stated, the infection was severe and the blood presented signs of grave anæmia—anisocytosis, a tendency to polychromasia and true basophilic punctation. A few normoblasts were also present. I put these appearances, at the time, down to the piroplasmosis, but in the light of Theiler's work one must reconsider this view.

Is there any possibility of these bodies being of the nature of the Jollykörper or Howell and Jolly bodies met with in embryonic blood, the blood of rodents, and in certain anæmic states? I think not, partly on account of their situation in the red cells (they tend to hug the margin so to speak), and partly because I have never seen Jolly bodies in process of budding. One may get two Jolly bodies lying near each other in one erythrocyte, but I do not think a Jolly body ever looks like a dividing coccus. Otherwise, however, I am bound to say that the ordinary spherical chromatin "marginal point" in size, shape, and colouring, cannot be distinguished from the nuclear remnants represented by Jolly's bodies.

On the other hand, Theiler's work is so careful and complete that there seems no reason to doubt the accuracy of his conclusions, especially as some of his observations have been confirmed by Spreull. Therefore, it would appear that in the Sudan, at

PLATE XVII



1–3 G. Buchanan

4–5 R. D. Muir

1. *Micro-filaria camelensis*
2. *Micro-filaria sanguinis equi Africani*
3. Micro-filaria of the hare

Leishman Stain × 600 *diam.*

4. Anaplasmosis of donkeys associated with *Nuttallia equi*. (*Vide also* Plate VI., fig. 18)

Leishman Stain × 1000 *diam.*

5. Parasite of Epizootic Lymphangitis *(Sarraja)* in equines in the Anglo-Egyptian Sudan (*Saccharomyces farciminosus*)

Leishman Stain × 1000 *diam.*

least, anaplasmosis may attack donkeys as well as cattle. What is the transmitter of the disease?

In South Africa, the blue tick, *Boophilus decoloratus*, is implicated. So far as we know, this tick does not occur in the Sudan, but *Boophilus australis* is present, having been found on cattle and dogs, and it is worth noting that Mr. King, our Entomologist, informed me that Professor Nuttall told him that there was a possibility that these two species will be found to be one and the same. The only tick found on the donkeys when they arrived in Khartoum was *Rhipicephalus evertsi*, but it is quite possible they may have harboured *B. australis* at Malakal, or wherever they became infected. Mode of transmission

Still, a different host, a different carrier, and possibly, also, a different species of parasite. It may be that this *Anaplasma* is not *A. marginale* and that another tick acts as its vector. It is hoped that opportunities may arise for working out this problem. Unfortunately, at the time the animals were here I did not pay much attention to the "marginal points," being interested in the *babesia* infection, and I expect I overlooked any special symptoms or post mortem appearances which might be attributed to gall-sickness. This shows how necessary it is to be constantly on the alert, to take nothing for granted, and to judge, so to speak, every case on its own merits. Of course, any disease in donkeys induced by the presence of *Anaplasma* may not necessarily be of the nature of gall-sickness. In slides of donkey's blood, recently sent him from the Bahr-El-Ghazal, Captain Archibald has also found *Anaplasma* and *Nuttallia equi* occurring together. It is probable, therefore, that one species of tick serves as the carrier of the parasites of both diseases in donkeys, and I should not be surprised if that tick turns out to be *Rhipicephalus evertsi*, so far as the Sudan is concerned.

Filariasis

Although filaria have been found in the Sudan in the blood of birds and reptiles I am not aware that, prior to a paper on a micro-filaria of the horse, which I contributed to the *Journal of Tropical Medicine and Hygiene*, these parasites, with one exception *(vide infra)* had been described in mammalian blood apart from human infection. They have come under observation in the blood of three animals, the horse, the camel and the hare.

In the Horse

On February 5, Captain F. U. Carr, Director Veterinary Department, sent me a pony which was suspected to be suffering from trypanosomiasis. Such was found to be the case, a trypanosome being present in fair numbers in the peripheral blood which, so far as can be told from morphology alone and a few negative inoculation experiments, is probably *T. nanum*. The pony was then treated with arsenic and was sent for re-examination on February 12, when trypanosomes were found to be very scarce, but filarial embryos were discovered in the blood. It is this micro-filaria which forms the subject of the present short paper.

History:—The owner of the horse, Mr. L. Landon, of the Sudan Irrigation Service, very kindly furnished me with the following particulars regarding his pony. It is an Abyssinian and was purchased in 1906 when three years old. Towards the end of 1908 it was taken to Uganda, reaching Gondokoro, in the Nile Province, about December 10 and proceeding thereafter as far as Kobe, at the north end of Lake Albert. At that time the pony was perfectly well, but was sent back to Nimule, on the Nile, and was there found, in March, to be slightly sick, but not to such an extent as to prevent its being ridden back to Gondokoro, brought down the Nile, and sent to Dongola. There it developed pneumonia in June or July and since that time has been more or less ill. Filaria in an Abyssinian pony

Present condition :—The pony has no marked signs of illness and is well-nourished. The blood coagulates rather more quickly than usual and is a little greasy, but not specially so. There are no skin lesions of any kind. As stated, filarial embryos were present in it. The blood in which they were found was derived from one of the branch veins of the ear and about one micro-filaria was present in each blood film—fresh or stained—examined, *i.e.* in about every small drop of blood.

Characters of the parasite

The parasite :—In the fresh blood the parasite is found to be exceedingly active, rapidly traversing and leaving the field of the microscope, twisting, coiling and lashing about incessantly, so much so that in a thickish film it causes a certain amount of destruction of the red cells. It possesses a blunt cephalic and very sharp caudal end. No evidence of a sheath can be seen. In cover-glass preparations it remains alive for at least six hours, though its movements become more sluggish and it rarely coils itself up as it does at first.

In ordinary preparations stained by the Leishman method and carefully measured by means of the ocular micrometer, it is found to vary in length from 115μ to 180μ. In citrated blood and in de-hæmoglobinised films this measurement becomes considerably less, *i.e.* 80μ to 95μ or thereby. Doubtless the medium or the process of staining causes some alteration. The average width is 4μ, but this also varies a little in stained films. These measurements were confirmed later by Bruce's method for trypanosomes.

The cylindrical body, especially in smears fixed in alcohol, is seen to be coarsely granular, and it presents, as a rule, four or five clear areas or spots. There is one at the head end, possibly of the nature of a cephalic prepuce as it varies in size and is not always visible. It is rounded, exhibits no hook or spicule, stains a faint blue, measures about 1·5μ in length and is continued into the central column of the cells as a narrow but very distinct slit about 3μ long by ·5μ broad. This does not show in all specimens and would seem to depend on what aspect of the parasite happens to be presented to view. In a parasite measuring 115μ in length the following spots were made out, apart from the clear area at the cephalic end and the accompanying slit :—

1. A well-marked somewhat oval bay, with centre 34·5μ from the anterior extremity and measuring 4·5μ × 1·5μ.

2. A small indefinite central spot with centre 73·5μ from the anterior extremity of the worm.

3. A large clear area with centre 90μ from the anterior extremity and measuring 6μ × 3μ but occupied in part by cell nuclei. This is the most characteristic and constant of the spots and the cell nuclei always seem to encroach upon it.

4. A small but distinct triangular caudal end, including the tip of the tail, which does not take on the stain and which is not seen in all specimens.

In other examples one has observed a small lateral bay between No. 3, and the tail tip and also a larger lateral bay, two-thirds of the way between the anterior extremity and No. 1 (Plate XVII., fig. 2).

The spots are not so evident in parasites observed in de-hæmoglobinised films or in smears of citrated blood. Examination with high powers reveals nothing of further note beyond well-marked annulations of the cuticle which are indicated in the drawing.

I unfortunately neglected to apply the special staining methods of Fülleborn and Rodenwald.

The only other filaria so far met with in horses in the Sudan, is a small nematode which I found in the crust of a skin ulcer sent me by Captain Williams, late of the Sudan Veterinary Department. He believed the condition to be Bursati.

A filaria has been discovered by Major Ensor in donkey's blood in the Bahr-El-Ghazal

but has not been fully studied, and in looking through the available literature one found that references to micro-filariæ in the blood of equines were rare.

Thanks to the courtesy of Mr. S. H. Gaiger, of the Indian Veterinary Department, I have been able to study Lingard's[1] paper, which gives a review of previous work, mentions Wedl's *F. equina* and Sonsino's *F. sanguinis equi* found in Egypt, Jakimoff's discovery of filarial embryos in horse's blood, Mazzanti's similar discovery in the hepatic vessels of a horse and a few other observations, but the whole is somewhat meagre, and, curiously enough, Hutyra and Marek more or less ignore the subject. Lingard's own work describes larval forms like those of *F. perstans* and *F. nocturna* which he found in the blood of horses in India and which, from his account, were evidently not unlike that under consideration.

Filaria sanguinis equi africani

It was not, however, until I came across an interesting and illustrated paper by Martini[2] that I could place this micro-filaria. Martini's paper, however, left little doubt but that the filaria in question was no other than *Filaria sanguinis equi africani* which he discovered in a barbary mare from Togo in the Berlin Zoological Gardens in 1902. His photo-micrographs of this filaria show that it is practically identical with that under discussion. Indeed, his Fig. 20 might almost have been taken from one of my slides. In his paper he points out that the presence of the filaria in his case was unassociated with illness or loss of weight, that the parasite measured between 100μ and 150μ in length by 4μ in breadth and that it possessed thick and thin ends. He describes its great activity and its mechanical destruction of red corpuscles, its longevity *in vitro* and its pale unstained areas, which closely correspond to those observed in the parasites of the pony from Uganda. He regards these spots as remnants of an embryonal membrane. He points out how his filaria differs from a pathogenic form previously found by Lange in the skin of the ear muscle of a horse and he points out its resemblance to *F. perstans* so far as size, activity and general appearance are concerned.

He did not find the parasite in blood from the smaller peripheral vessels but only in defibrinated blood taken from the jugular vein. It occurred in comparatively small numbers, about one to every five drops of blood taken.

This, however, and the fact that he described the body as finely granular are the only points of difference I can perceive between his parasite and that under discussion, and, considering that the animal in which he discovered it was living under very dissimilar conditions to those obtaining when it became infected, one cannot lay any stress on such variations.

I think that this parasite is *Filaria* or, better, *Micro-filaria sanguinis equi africani* and, so far as I can find out, it is the second occasion on which it has been observed.[3]

Probability of infection while in Uganda

The infection was probably acquired in Uganda and is not of a pathogenic nature, the chief interest attaching to it being possibly the somewhat close resemblance of this embryo blood worm to the larval *F. perstans* which is such a common human parasite in Uganda. That it is not *F. perstans* is evident enough. It possesses no retractile spicule, it is smaller, its caudal end is not truncated and its spots do not correspond to those of the *perstans* embryo. Still it would appear to be a parasite of the *perstans* class, and doubtless the adult forms exist in situations similar to those in which the male and female *perstans* were found by Daniels.

[1] Lingard, A. (1905), *Observations on the Filarial Embryos found in the general circulation of the Equidæ and Bovidæ, and their probable Pathological Significance.* London.

[2] Martini, E. (1903), "Ueber eine Filaria sanguinis equi," *Zeitsch. für Hyg., etc.* Vol. XLII., pp. 351-352.

[3] Mandel has quite recently described a very similar blood-filaria in a horse in Europe. *Cent. f. Bakt.*, Orig., December 17, 1910, Vol. 57, No. 1.

One may conclude by noting that on both occasions when the blood was found to contain the parasites the examination was made about mid-day, and that gerbils, a jerboa and a young rabbit inoculated with blood from the pony, in connection with work on the trypanosome, have failed to exhibit the filaria.

In the Camel

In an article on Debab, the camel trypanosomiasis of Algeria, the Sergents[1] mention the frequent presence of micro-filariæ in the blood of camels but they do not describe them. Mason[2] has found these parasites in the peripheral blood of camels in Egypt. He does not describe them either, but mentions their apparent periodicity, as they were more easily found in smears taken late in the afternoon or at night. He also mentions the discovery in the testicle of camels of what may be the parent worms. What he took to be a female was 20 cm. long, while the males were 12 cm. in length and possessed tails curved in a spiral manner. These worms were found in a bunch in the central vein of the organ. In the Report of the Government Bureau of Microbiology of New South Wales for 1909, an account is given of filaria embryos found in the blood of camels in Western Australia. They appear to have been the embryos of the Indian *F. evansi*.

A Sudan case

In March, 1910, I found micro-filariæ in films of camel's blood sent me by Mr. E. T. von Becker, of the Sudan Customs Department, from Korara, in the Red Sea Province. Being a microscopist, Mr. von Becker had himself noted the presence of worm-like bodies in the films but was not able to diagnose them, though he was well up in trypanosome infections.

Symptoms.—According to Mr. von Becker these are quite unlike those seen in camels suffering from trypanosomiasis and are as follows :—

1. Loss of appetite.
2. Roughened and staring coat.
3. Watery secretion from the eyes.
4. Œdema of the external genitals.

At the time the sick animal was examined it had been ill for 9 months. It was stated that there had been temporary improvements but latterly a rapid decline, the camel being very weak and emaciated.

Morphology of the parasite

The parasite.—No opportunity was afforded me of studying this micro-filaria of the camel in the living state, but Mr. von Becker describes its movements as rapid and snake-like. Fig. 1 on Plate XVII. gives a good idea of it in stained preparations, though, as a rule, the food material, so clearly shown, is not quite so much in evidence albeit constantly present in all specimens examined. There was about one parasite per film. The length varies from 220μ to 237μ and it will be seen that the cylindrical body shows definite clear areas or spots. In a parasite measuring 220μ the positions and sizes of these areas were as follows :—One, at the cephalic extremity, 10μ in length and occupying the whole breadth of the body, a second, with irregular margins, 45μ from the cephalic end and running obliquely across the whole body thickness ; a third, roughly oval in shape, 65μ from the anterior extremity and taking up three-quarters or the whole of the body breadth ; a fourth, of irregular shape, stretching nearly wholly across the parasite and some 30μ from the sharp tail tip.

[1] Sergent, Ed. and Et. (January, 1905), "Trypanosomiase des dromadaires de l'Afrique du Nord." *Ann. de l'Inst. Past.*, Vol. XIX., p. 17.

[2] Mason, F. E. (June 30, 1906), "Filariæ in the blood of Camels in Egypt." *Journal of Comparative Pathology and Therapeutics*, Vol. XIX., p. 118.

It is scarcely worth while giving the dimensions of these areas, as I find they vary in size and also in appearance. They appear, however, to be constant in occurrence and in position. There is also what may be called a clear area at the caudal end, for the column of cell nuclei terminates some little way from the tail tip in all the specimens examined. In one specimen I have also seen a tiny central gap between number four and the end of the column of cell nuclei. These latter are not nearly so distinct as in the micro-filaria of the horse, the general aspect being more homogeneous, but this may merely be a staining effect. With high powers, the annulations of the cuticle, seen in the case of the horse parasite, are not visible. I have had no opportunity of staining by any of the special methods, so cannot enter more fully into anatomical details. The blood showed a very marked leucocytosis, the increase being chiefly in the large lymphocytes. I append a differential count of 500 cells.

Eosinophiles	2·6 per cent.
Polymorphs	32·8 ,,
Large Mononuclears	3·8 ,,
Large Lymphocytes	40·6 ,,
Small Lymphocytes	19·2 ,,
Transitionals	1·0 ,,
	100·0 per cent.

A few normoblasts were present.

The adult form not found

I did not succeed in obtaining the adult nematodes which very probably have their habitat in the testicles. It would be interesting to know if this is the micro-filaria found by Mason in Egypt, and also to determine the carrier. The most common ecto-parasites of the camel are *Hippobosca camelina* and *H. maculata*. *Lynchia exornata* has also been taken on a camel, but this was probably accidental.

Amongst ticks may be mentioned *Hyalomma ægyptium*, *Amblyomma variegatum* and *Rhipicephalus sanguineus*. Mr. King informs me that, in the Red Sea Province, the following diptera bite camels: *Stomoxys sp.*, *? Lyperosia minuta*, *Tabanus sufis* and three new and undescribed species of *Tabanidæ*, and *Pangonia rüppellii*. It is, of course, probable that mosquitoes also suck the blood of camels in this region and that they are the vectors.

I suggest the provisional name of *Micro-filaria camelensis* for this larval nematode of camels which seems to be of considerable economic importance.

In the Hare

In May, 1910, I shot a hare at Jebelein, on the White Nile, which, Mr. Butler informs me, was in all probability *Lepus hawkeri*. In its blood I found micro-filariæ present in considerable numbers. The parasite is smaller than either of the foregoing, being about 95μ in length. Fig. 3 on Plate XVII. shows its general appearance when stained by the Leishman method. There is a clear area 5μ in length at the cephalic extremity, two lateral bays, one 40μ from the anterior end, the other 10μ from the rounded tip of the blunt tail. In addition, there is a central clear area reaching across the whole, or nearly the whole, of the body breadth and situated about 60μ from the anterior end. In some, no clear area is visible at the rounded cephalic extremity, while others have a

small additional bay or central spot about the middle of the anterior third of the body. The column of cell nuclei is coarsely granular and reaches right to the caudal end. No annulations of the cuticle have been noted. The parasites were numerous in the blood, two being not uncommon in one thin film, low power field (oc. 1; obj. 4 Leitz), while as many as three may occur.

I found two, presumably parent worms, in the abdominal cavity and sent them to Dr. Leiper at the London School of Tropical Medicine.

Parasites

A considerable number of intestinal worms has been collected from cattle, sheep, goats and other animals in the Sudan. Some of the material was sent to Professor Looss, some to Dr. Leiper.

Acid-fast Bacilli in the Lung of a Camel*

by

Captain R. G. Archibald, M.B., R.A.M.C., attached E.A.

Pathologist and Assistant Bacteriologist

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In the Sudan, human tuberculosis is an extremely frequent pathological condition, while the same disease occurring in mammals cannot be considered a common one. Dogs and captive monkeys seem to be more susceptible to it in the Sudan than any other animals. A short time ago Captain A. G. Cummins, R.A.M.C., sent to the laboratories a lung which he had removed from a camel that had died in Kordofan.

Macroscopic lesions

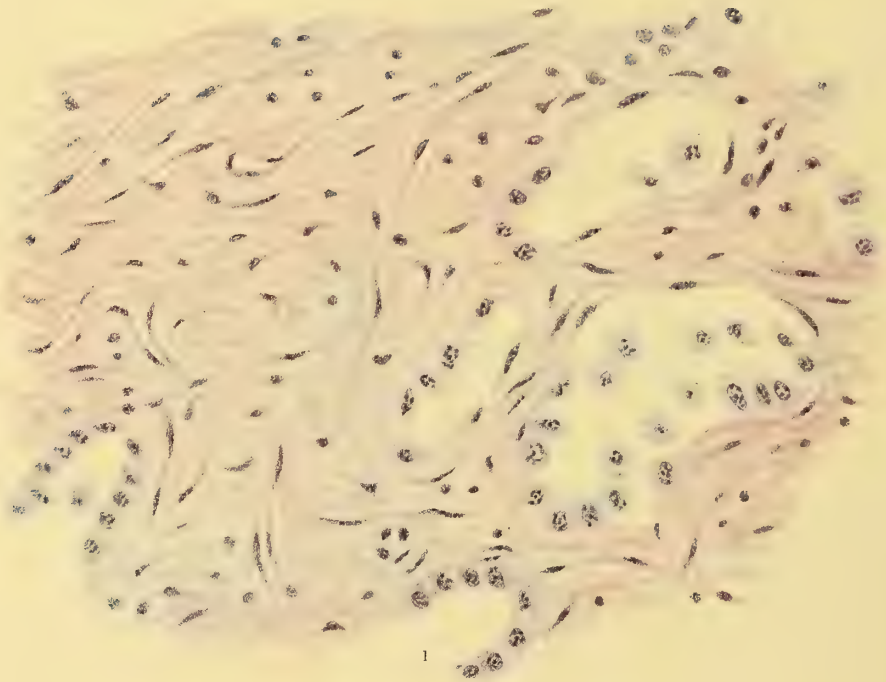
The gross macroscopical lesions present were as follows:—Scattered throughout the surface of the lung substance were numerous grey fibrotic-looking masses about the size of millet seeds. On palpation, these were firm in consistence, and distinctly raised above the surrounding lung tissue. At the base of the lung there were two or three large caseous-looking areas measuring about an inch in diameter and sharply demarcated from the surrounding tissue. On incising these caseous-looking masses they were found to be quite firm and fibrous in consistence. Most of the pleura was adherent but did not show the presence of any nodules on its visceral layer. Various incisions were made through different parts of the lung substance, but in no case were cavities detected, nor was there any marked enlargement of the bronchial lymphatic glands.

For the purpose of microscopical examinations, sections were made through the diseased parts of the lung, fixed, and stained by Van Gieson's and Ziehl-Nielsen's methods.

In sections stained by the former method, and examined microscopically, it was at once apparent that the lesions represented a type of miliary tuberculosis in which reparative processes in the form of a fibrosis were present.

* A preliminary report appeared in the *Journal of Comparative Pathology and Therapeutics*, March 31, 1910.

PLATE XVIII

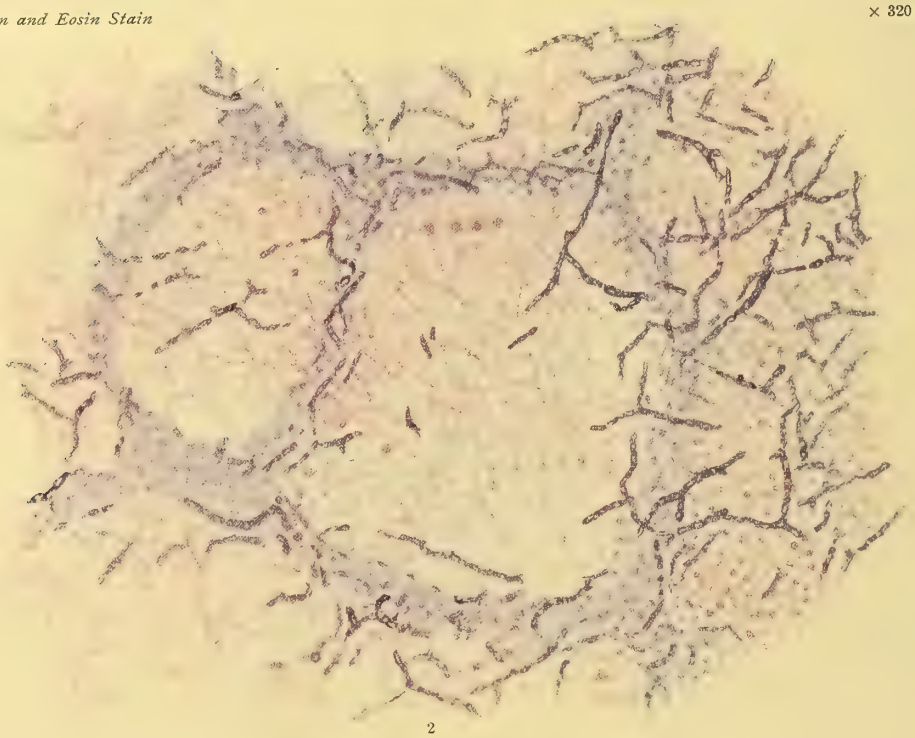


1

R. D. Muir

1. Interstitial pneumonia in Lung of Camel

Hæmatoxylin and Eosin Stain × 320 *diam.*



2

R. D. Muir

2. Aspergillary pneumokoniosis in Lung of Turkey

Gram-Weigert Stain × 300 *diam.*

Microscopic appearances

The millet seed areas represented caseous tubercles undergoing a connective tissue infiltration. They consisted of necrosed tissue in which connective tissue cells were present, and were surrounded by endothelioid cells, irregular in shape, and containing one or more nuclei; they in turn were surrounded by round cells of an inflammatory type. The air vesicles in the vicinity of the tubercles showed evidence of collapse, but there was no marked catarrhal or croupous exudate present. The tubercles were not confined to the interlobular septa but were present throughout the lung substance. The large caseous fibrotic areas met with at the base of the lung represented masses of necrosed tissue which had undergone complete fibrosis as shown by the large numbers of connective tissue cells present. In these areas all traces of the normal lung substance had disappeared. Typical giant cells were also in evidence.

In sections stained by Ziehl-Nielsen, acid-fast bacilli were found to be present in the vicinity of the tubercles. These acid-fast bacilli occurred as single, beaded rods with rounded ends measuring on an average 4 μ, and morphologically resembled tubercle bacilli.

Conclusions

From the results of the examination of this specimen one is inclined to consider that this was a pathological condition resulting from the effects of an acid-fast bacillus morphologically identical with the tubercle bacillus. The macroscopical lesions simulated those of acute miliary tuberculosis followed by a reparative fibrosis.

In the literature at one's disposal no reference to such a condition occurring in the camel has, as far as one knows, been previously recorded,[1] and it is difficult to conceive how such an animal is a suitable subject for a disease like lung tuberculosis when one considers its open-air existence and that its life is rarely spent in the vicinity of towns. Mr. Esmond Brown, M.R.C.V.S., who for some time was Director of the Government Abattoir in Cairo, stated, in a letter to me, that he had frequently seen lesions of a tubercular type in the lungs, liver and kidneys of old camels, and in a few cases acid-fast bacilli were found to be present in these lesions. He further stated that the fibrous nature of these lesions appears to be a feature of the disease in camels.

Interstitial Pneumonia in a Camel's Lung

by

Captain R. G. Archibald, M.B., R.A.M.C., attached E.A.

Pathologist and Assistant Bacteriologist

Wellcome Tropical Research Laboratories, Khartoum

Plate XVIII., fig. 1

In this specimen the pathological changes represent a contrast to those present in the camel's lung in which acid-fast bacilli were found.

[1] See, however, a recent paper by Leese in the *Journal of Comparative Pathology and Therapeutics*, December, 1910.

The specimen was sent by Captain Knott, A.V.D., and was taken from a camel that had died in the Red Sea Province. No notes on the history of the camel's illness were obtainable.

The gross macroscopical lesions present were as follows :— In appearance, the lung was of a greyish colour and only slightly crepitant. In consistency it was extremely hard, and present on its surface were a few small granular areas of yellowish-white colour.

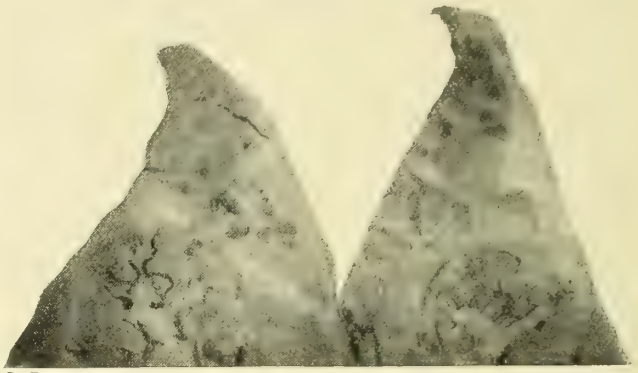


Fig. 106.—Fibroid pneumonia in a Camel's lung.

Sections were fixed and stained, and microscopical examination showed that almost the whole of the alveolar tissue of the lung had disappeared and had been replaced by a fibrous tissue, which in parts was of a dense nature. In some areas the alveolar walls could be more or less clearly defined, limiting a fibrinous exudate, in which leucocytes and young connective tissue cells were present. The alveolar walls were considerably thickened, and infiltrated by young connective tissue cells, which could be seen penetrating the contents of some of the alveolar sacs, thereby causing a complete organisation with the result that almost the whole of the lung was represented by proliferative changes of a fibroplastic type. Even the blood-vessels and the capillaries shared in this, and many of the latter were almost obliterated.

Fibrotic changes in the lung

The small yellow granular areas were represented, microscopically, by patches of necrosis in which the alveolar walls were considerably broken down. Leucocytes and round cells of an inflammatory type were present in these areas. The walls of the bronchi and bronchioles were thickened. A small amount of free blood pigment was present. Tubercle nodules were not present nor were any giant cells seen. Sections were stained by Ziehl-Nielsen's and by Gram's methods for the purpose of demonstrating tubercle or other bacilli, but the results were negative.

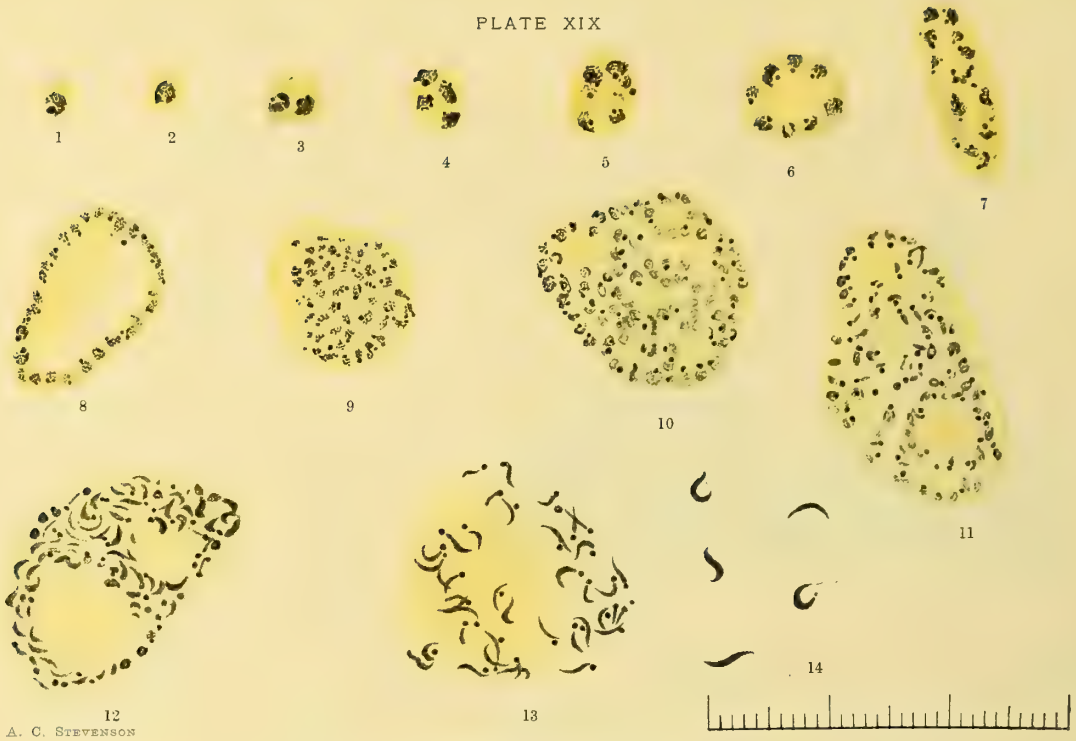
Conclusions

From the macroscopical and the microscopical evidence one must conclude that this condition represented the somewhat uncommon one of interstitial pneumonia in which most of the alveolar tissue of the lung had been replaced by fibrous tissue. As far as I know, this pathological entity has not been previously described in camels. As a primary lesion in man, interstitial pneumonia generally exists in the form of a pneumokoniosis; syphilis also causes an interstitial pneumonia. More commonly it is a condition either secondary to an acute croupous pneumonia or to a tuberculous infection.

Malkmus[1] has recently mentioned it as a sequela of contagious equine pneumonia.

[1] Malkmus, Dr. C. (December 31, 1909), "Contagious Equine Pneumonia." *Journal of Comparative Pathology and Therapeutics.*

PLATE XIX

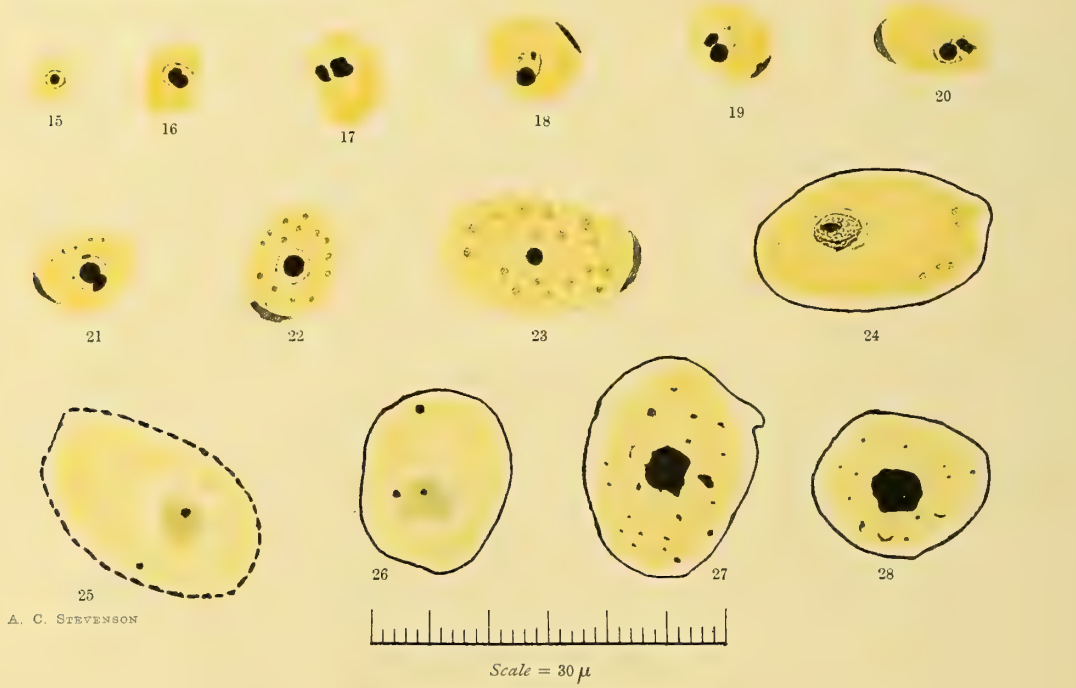


A. C. Stevenson

Microgamete Formation

1—3. Early forms of microgametocyte showing division of karyosome
4—9. Gradually enlarging forms with repeated division of nucleus and karyosome
10. Commencing condensation of chromatin granules
11—12. Lengthening out of condensed chromatin to form body of microgamete
13. Adult microgametes with karyosome still apparent
14. Free microgametes with terminal flagellum

Iron Hæmatoxylin and Orange Stain



A. C. Stevenson

Macrogamete Formation

15—17. Early forms showing primary division of chromatin of nucleus
18. Cap of extruded chromatin seen flattened between parasite and containing cell
19—21. Secondary division of chromatin of nucleus
22—23. Commencing formation of granules in protoplasm after nuclear division
24—26. Adult macrogametes awaiting fertilisation. In 25 capsule is not yet completed
27—28. After fertilisation showing irregular chromatin staining granules in cytoplasm

Iron Hæmatoxylin and Orange Stain

Coccidiosis of the Intestine in the Goat

BY

A. C. Stevenson, M.B., M.R.C.S., L.R.C.P. (Lond.), D.P.H. (Cantab.)

While working in the Wellcome Tropical Research Laboratories at Khartoum in the winter of 1908–9, I came across the above condition. The goat was one that had come from higher up the Nile and had been sent to the laboratories for diagnosis. Dr. Balfour was away, and, unluckily, notice was not given me of the goat's death till well on in the day, so that the post mortem took place about 9 hours after death and in the afternoon. The goat, I learnt, had had great difficulty over defæcation, though from the condition of its stomach it was feeding well.

Post mortem.—Skin very scaly, condition resembling favus. Thoracic organs normal, liver, spleen, kidneys, etc., normal. A fair amount of fluid in the peritoneal cavity, with adhesions recent and old. Numerous encysted cysticerci present in outer wall of gut, some calcareous. The upper part of the small intestine was very translucent, and showed numerous small white patches varying from 1–3 mm. in diameter. Portions of the gut with nodules were hardened in formalin and showed coccidia in the later stages of sporogony, the capsule being formed, and many microgametes free. The intestinal epithelium was practically non-existent, but most of this was probably due to post mortem changes.

Loss of intestinal epithelium

The second goat was one that also seemed ill, and, externally and internally, the conditions were much the same as in the first, except that the colonies in the gut were fewer in number, and the gut in this case was not denuded of its epithelium. Here sporogony had not gone so far, the coccidia seldom having capsules, but the growth of gametocytes was well shown. Tissue was fixed in corrosive sublimate and glacial acetic acid.

The third goat was a younger one, and did not show any marked signs of illness. The condition of favus was not so marked, and the colonies of coccidia were again fewer in number. Here again, however, only early sporogony phases were to be found, and those mostly microgametocytes. The mucous membrane was intact.

The colonies of coccidia are very well defined, very few individuals being found infecting cells away from the main mass. So definite is this that the cells on one side of the lumen of a gland may be infected while those on the other are not.

Clearly defined distribution of the coccidia

I have later received from Dr. Balfour portions of the intestines of two other goats, and also their fæces. The first of these, Dr. Balfour tells me, was distinctly ill, the second, however, showed no symptoms. In both of these, coccidia were present, but only in late stages, many having their capsules formed. The mucous membrane of the small intestine was in both cases entirely gone, though in the portions of large intestine examined it was still present. There were also some enlarged lymphoid follicles with formation of scar tissue in the centre, and in the large intestine under the mucous membrane there were small round cell masses, each containing a small nematode worm. The lymphoid patches in the small intestine closely resembled those in a human case from which Dr. Balfour sent me tissue some time ago. Search for coccidia in the fæces was disappointing. Very few were found and those that were found showed no signs of division, being practically in the same state of development as the later stages seen in sections of the intestine.

Schizogony Cycle

No coccidia in this stage of development have been found in any of the tissues. Professor Minchin suggests that this stage takes place before any symptoms are seen in the animal. From what I learn from Dr. Balfour, the second of the goats, of which portions of the intestines and the fæces were sent home to me, appeared perfectly well, though the coccidia were far advanced in development in the sporogony stage.

Probably a double type of schizogony

It would appear, however, that there is a schizogony of a double type, *i.e.* that at a very early stage there is a differentiation of sex amongst the merozoites and that we get macro- and micro-merozoites going on through several generations.

I am led to this belief by the fact that in any one colony in the gut there is always a great excess of one sex, and in several colonies I have found nothing but microgametocytes, those nearer the lumen of the gut being older than those in the deeper portions of the glands.

Further, I find that in sections of liver from a rabbit infected with coccidiosis, there are unquestionably two types of schizonts present. The one produces large merozoites with a well-marked nucleus showing a well-marked karyosome, closely resembling the young macrogametocyte described on *page* 358. The other type of schizont divides into small merozoites which are more numerous than the above, and which have a nucleus with a small karyosome and numerous small granules of chromatin as in the young microgametocytes described later. This double schizogony has been described in *Adelea ovata.*

Sporogony Cycle

Microgamete Formation (Plate XIX., figs. 1–14)

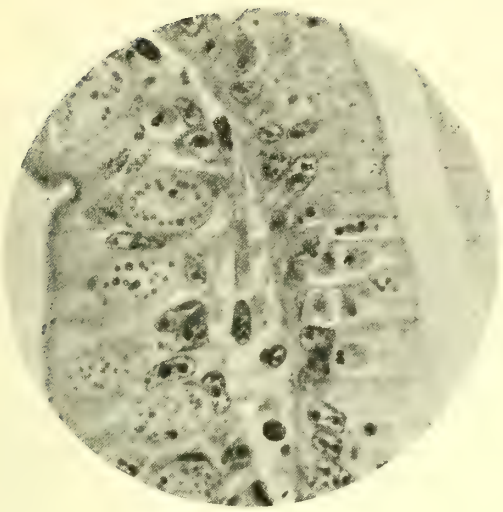
The microgametocyte is first seen in the cell as a small body about 3–4μ in diameter, with an indefinitely outlined nucleus containing small granules of chromatin and one larger one, the karyosome, generally a little separated from the others, which are arranged in a crescentic manner on one side of it. The karyosome divides first and this is followed by the division of the remainder of the nucleus. Frequently, division is more rapid, the karyosome dividing twice before division of the rest of the nucleus takes place. Concurrently with this the protoplasm of the microgametocyte increases in amount. Division goes rapidly on, the nuclei arranging themselves around the periphery of the microgametocyte until a large body is formed often 30μ in the longest diameter, and the number of nuclei is often so great that the surface becomes involuted to accommodate them all. The size of the adult microgametocyte, and the number of nuclei present in it, vary considerably at the time the final changes begin to take place. The only apparent reason for this irregularity seems to be the varying amount of pressure exercised by the surrounding tissue and parasites.

The microgametocyte

When division of the nuclei has stopped, the small granules of chromatin in each nucleus condense into a small ovoid body with the karyosome slightly separated from it at one pole. This arrangement of the karyosome is much better seen in sections stained by Twort's or Giemsa's stain, than in those stained by iron hæmatoxylin (Plate XIX., fig. 13, and Plate XX., fig. 4). These ovoid bodies gradually lengthen out into thin spindle shapes, and are all arranged round the mass or masses of residual protoplasm. Whether there is one centre or more depends on the frequency of division of the nuclei of the microgametocyte and the subsequent involution of the surface.

The adult microgamete has a terminal flagellum and a vacuole situated about a third of its length from the one end of the body. The karyosome seems to disappear, but may possibly reappear as the flagellum. I have only seen the vacuole definitely in the tissue lately sent home by Dr. Balfour, and even there it does not appear readily

PLATE XX



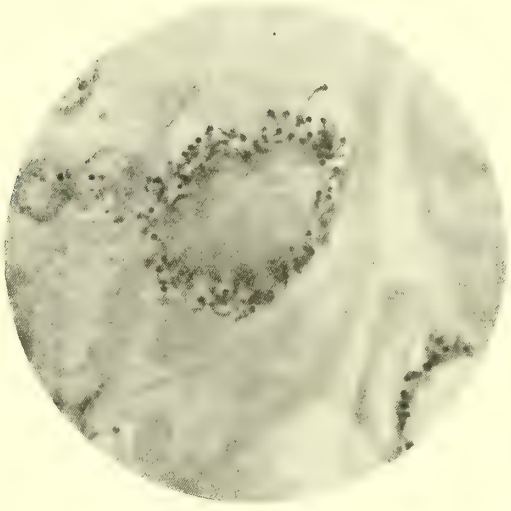
1



2



3



4

A. C. Stevenson

Coccidiosis of Intestine in Goat

1. Portion of villus with nearly every cell infected. On the right, young macrogametocytes with two young microgametocytes about centre. On the left, nearly adult macrogametocytes.
2. Macrogametocytes and one microgametocyte with nearly adult microgametes in centre.
3. Two microgametocytes showing arrangement of microgametes around two centres of residual protoplasm. Also two macrogametocytes with cyst already formed round them while still in host cell, evidently before fertilisation as karyosome is still present.
4. Nearly adult microgametes showing karyosome still attached to one end.

Photographed with Zeiss 2 *mm. lens and camera length of* 50 *cm. Figs.* 1, 2 *and* 3 *with No.* 4 *compensating ocular and fig.* 4 *with No.* 8 *compensating ocular.*

with iron hæmatoxylin staining. It was best observed by staining with Ehrlich's hæmatoxylin and eosin (fixation various). As my drawings were finished before I received this last tissue, the vacuole is not shown.

Macrogamete Formation (Plate XIX., figs. 15–28)

The youngest form of the macrogametocyte observed is considerably larger than that of the microgametocyte, being 5 μ–6 μ in its longest diameter, and the protoplasm stains more readily with acid stains than that of the microgametocyte. The nucleus has a marked karyosome, but except in the earliest stages there is very little other chromatin present. A very interesting peculiarity of the chromatin in the macrogametocyte is that it seems impossible, in tissue fixed in corrosive sublimate and glacial acetic acid, to stain it with basic dyes, such as the blue in Giemsa's stain and the neutral red in Twort's stain, though it stains readily with iron hæmatoxylin (Heidenhain). The chromatin in the microgametocyte is readily stained by the basic stain in these two preparations.

Staining peculiarity of the chromatin of macrogametocyte

The karyosome at any early stage divides, and one-half is extruded from the cell nucleus and cell, and is often seen as a cap of chromatin flattened out between the parasite and its containing cell, while the remaining portion divides again. The one part of this is again extruded or absorbed, while the remainder persists as the karyosome of the adult macrogamete.

The macrogametocyte enlarges, and, soon after the second division of the karyosome, granules appear in the protoplasm, at first small but eventually growing much larger. These granules retain iron hæmatoxylin stain well, though not so markedly as the karyosome. They stain deeply with the acid stain in Giemsa and Twort.

Enlargement to full size, 26 μ–28 μ × 17 μ–18 μ, goes on without much further change, except that the nucleus becomes enlarged and vesicular with the single marked karyosome remaining. The granules in the protoplasm also become larger and more marked, when nearly adult capsule formation commences. Granules are secreted which stain readily with iron hæmatoxylin, much less readily with Delafield or ordinary hæmatoxylin, and not at all with either the basic or acid stains in Giemsa or Twort. These granules arrange themselves round the periphery of the macrogamete, appear to be extruded and then to flatten out on the outside of the parasite, openings being left between the various plaques thus formed which fuse at first in places, then altogether except for one opening left at one end of the long diameter of the cyst wall to form a micropyle. A cone of attraction appears at one end of the parasite which is directed towards the opening in the cyst.

Fertilisation

Fertilisation apparently takes place at this stage from the change in the character of the nucleus, though I have been unable to find direct proof of the entrance of the microgamete. In the apparently unfertilised macrogamete, the nucleus possesses but little chromatin besides the karyosome, whilst, apparently after fertilisation, the whole nucleus becomes a dense mass, staining strongly with iron hæmatoxylin and irregular in outline. At this stage, too, there are seen in the protoplasm numerous small irregular masses staining like chromatin. Are these portions of the karyosome expelled from the nucleus as described by Schaudinn, or are they remains of numerous microgametes which have entered the macrogamete and become degenerate as described by the same observer in *Cyclospora caryolytica* in the mole, which this coccidium seems closely to resemble in its sexual development? There is no definite evidence.

As mentioned above, I have not been able to follow the matter further, and so have no knowledge of the number of spores or sporozoites formed. All that I have seen in examining the fæces are a few coccidia in the same stage of development as the most advanced of those in the intestinal mucous membrane.

PLATE XXI



A. C. Stevenson

PROTOZOA IN BLOOD OF *Bufo regularis*

1–8. *Trypanosoma rotatorium.* 1–3 show the rolled-up condition of large forms, the shape usually seen in fresh specimens; 4 and 5 show unrolled forms; 5 shows arrangement of myonemes; 6–8 show small forms.

9–14. *Hæmogregarina tunisiensis.* 9–13 show space in capsule which stains red with Romanowsky stain and black with Iron Hæmatoxylin.

15–19. *Drepanidium magnum.*

20–25. HÆMOGREGARINES IN THE BLOOD OF *Trionyx nilotica.* 25 is possibly a schizogony form from the lung.

Giemsa Stain. The green of the blood corpuscles in figs. 15–18 is due to over-fixation in osmic vapour.

Pathogenicity

Coccidiosis in the intestine of cattle, goats, etc., has been described in Central Europe. There, however, it was seldom fatal except in young animals. I have now considerable doubt, however, whether the coccidia will account for the illness of some of these goats. The stripping of the entire mucous membrane of the small intestine to the very depth of the glands, except in those places where coccidia are, the enlargement of the mesenteric glands, which show marked increase of fibrous tissue and centres of cell degeneration, and the fact that advanced stages of coccidiosis give no symptoms, seem to point to some more acute cause than coccidiosis, unless there is the formation of some virulent toxin.

Pathogenicity is doubtful

I regret I have not enough data to definitely place the organism. It is probably the same as that affecting cattle in East Africa, as I have lately heard, and possibly the same as that in Central Europe. As mentioned on *page* 358, its sexual development seems closely allied to that in the intestine of the mole, though its habitat is not in the nucleus.

A few Notes on the Protozoa Parasitic in Bufo regularis in Khartoum

BY

A. C. Stevenson, M.B., M.R.C.S., L.R.C.P. (Lond.), D.P.H. (Cantab.)

Protozoa in the Blood

Trypanosoma rotatorium

In examining fresh specimens of the above toad's blood in Khartoum, I found *Trypanosoma rotatorium* present, without extensive search or cultivation in each instance, in 6 cases out of 21. The period of examination was from the middle of November to the middle of February.

I had at first considerable difficulty in recognising the organism in the fresh state, as in that condition its appearance when adult is quite different from that seen under similar circumstances when search for the commoner mammalian trypanosomes is made.

The trypanosome is seen as a small body, roughly about the same length in longest diameter as the red blood corpuscle, resembling the flower of the convolvulus in shape, and rapidly rotating on its long axis. Its flagellum can be seen flickering beyond what would be the edge of the petals of the flower. The posterior end of the parasite, or what would be the base or narrow end of the flower, is generally applied to one of the red blood corpuscles. Though I know this was the first trypanosome described, I have never seen or heard mentioned the reason of the name.

By emulsifying blood in citrate of soda solution, fixing with osmic acid vapour, spreading gently with a needle and drying, I was able to stain the parasite in this condition (Plate XXI., figs. 1–3). Spreading films in the ordinary way generally leads to their being unrolled or crushed.

Method of making and fixing films

An interesting point which appears in some of the unrolled and stained specimens is the arrangement of the myonemes. Each myoneme appears to start from near the posterior end of the body, about half-way to the edge of the undulating membrane, to which they radiate, each divides into two, and each half coalesces with the half from the myoneme on each side of it, except in the case of the first and last, where the anterior and posterior halves of each respectively remain alone (Plate XXI., fig. 5).

Arrangement of the myonemes

From this arrangement a stimulus started in one myoneme may be transferred to the next, and thus undulatory movement of the membrane may be kept up.

Hæmogregarinæ

Two forms occur very commonly in this toad. One form or both were present in 17 out of 21 cases. The period of examination was the same as above. They were both far more common in the blood in the earlier part of my stay in Khartoum than in the latter, and as I never found any signs of schizogony in any of the organs, I think it is a fair assumption that this process only takes place in the summer months in cold-blooded vertebrates. I believe that the schizogony stages of similar parasites in lizards found by Dr. Wenyon were in specimens collected in the warmer part of the year. These stages in warm-blooded animals, the jerboa for instance, seem to take place all the year round.

Hæmogregarina tunisiensis

The first, and if anything the commoner form of these two hæmogregarines, is found in the blood corpuscle in a firm cyst wall, and is sharply bent upon itself about the middle. The cyst wall is double at the end at which the parasite is bent, and the space between the two layers contains a substance which stains a bright red with Romanowsky stains and retains the iron hæmatoxylin stain well (Plate XXI., figs. 9–11 and 13). The cyst is not as long as the blood corpuscle. Observed in citrated blood, or in a similar emulsion of spleen pulp the parasite, especially in the latter situation, is often seen free (Plate XXI., figs. 12 and 14). Its movements are fairly rapid, and of a definite gregarine type, contractile waves passing down the body being seldom seen until motion has practically ceased. When the motion begins to slow down, the mucoid thread secreted by the organisms becomes visible, and, after forward motion ceases, this thread has been seen to become corkscrew-shaped. Movement has also been seen in the cyst, the parasite completely turning round inside it. Average size of the cyst is $14\mu \times 8{\cdot}8\mu$.

This parasite is probably the same as that observed in a frog in Tunis, and has been named *Hæmogregarina tunisiensis*.

Drepanidium magnum

The second and larger hæmogregarine is probably *Drepanidium magnum* (Plate XXI., figs. 15–19). It lies apparently free in the blood corpuscle, of which it fills the entire length, its two ends being slightly bent over. Size in corpuscle $23\mu \times 7\mu$. In films in which the blood corpuscle has disappeared a stained capsule is visible, and, in citrated blood, the ends of the organism are generally still bent as if enclosed. I have never seen this form actually motile, but possibly the large free organism shown (Plate XXI., fig. 18) is of this type. Both forms show a marked rostrum.

On staining tissue fixed with saturated corrosive sublimate and glacial acetic acid, the nucleus of both these hæmogregarines is found to be that of the definite gregarine type mentioned by Professor Minchin, *e.g.* a vesicular nucleus containing granules of chromatin on the fibres of a fine network. In these cases there are smaller granules round the periphery with generally four larger granules in the centre.

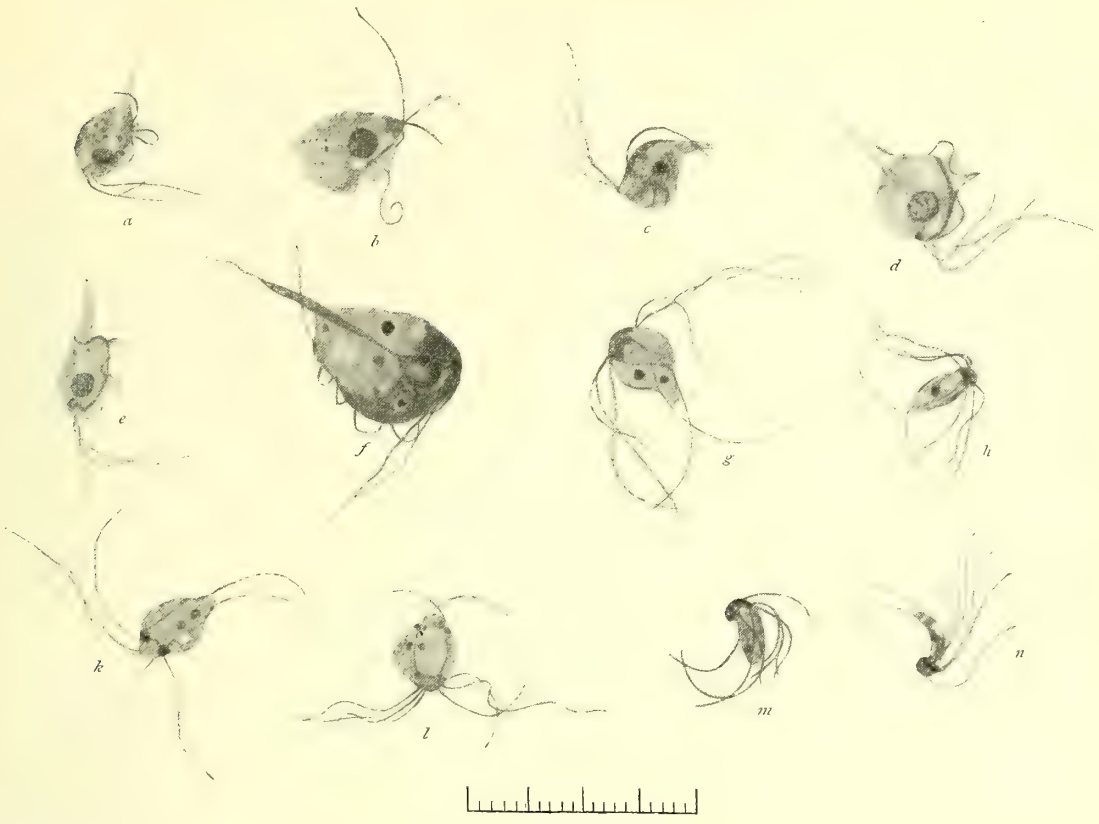
I have shown in the drawing some hæmogregarines from the river turtle (*Trionyx*) of the Nile (Plate XXI., figs. 20–25). The specimen was a young one. I also show a possible schizogony stage of this from a film from the lung (Plate XXI., fig. 25), but as it was the only one I found, I do not place much reliance on it, and, in addition, the films were hurriedly made.

Protozoa in Intestine

Flagellates

Flagellates

Trichomonas and *Hexamitus* (*Octomitus*) are seen very commonly in most cases (Fig. 107). They show no difference from those described by Dobell in the frog in this country or from those described in the mouse and rat by Wenyon, where I have also observed them.



A. C. Stevenson

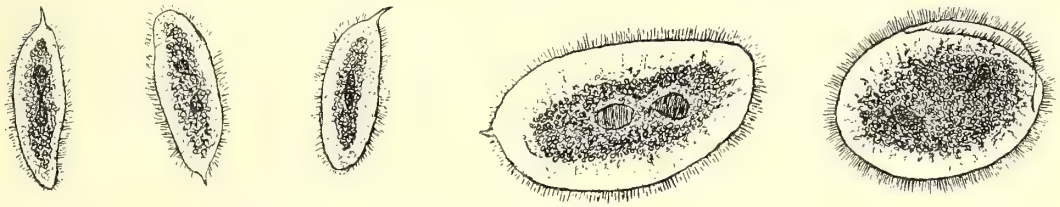
Scale = 20 μ

Fig. 107.—Flagellates in Intestine of *Bufo regularis*

a–f.—Trichomonas batechorum *g–n.—Octomitus* (*Hexamitus*) *dujardini*

Ciliates

Nyctotherus cordiformis and a *Balantidium* are both common, but instead of Opalina we have another organism something of the same class though it differs in its nuclear arrangement. The largest specimens of this are about 80μ by 60μ, while the smallest are Ciliates



A. C. Stevenson

× 450 *diam.*

Fig. 108.—Unnamed organism in *Bufo regularis* (Khartoum Toad)

about 60μ by 16μ. They are covered by cilia of a uniform length, have no apparatus for ingestion or egestion, and the nucleus usually consists of two pear-shaped bodies connected by a definite narrow strand (Fig. 108). Up to the present I have not identified this. It is characterised by having a definite spine at one end, especially in the smallest and middle-sized forms.

I cannot conclude without expressing my deep thanks for the help and kindness I received from Dr. Balfour, Mr. Currie, and any official of the Sudan Government, from the Sirdar downwards, with whom I came in contact.

Miscellaneous Notes

by

The Director

Hæmatozoa

One has again to record the presence of *Hæmoproteus* (*Halteridia*) in the blood of birds. Those in which they have been found since the date of the last record are—

Dendrocygna viduata—the whistling teal
Sarcidiornis melanota—the comb duck ... } On the Upper White Nile.

Ardetta melanota—the little bittern ... by Captain Fry in specimen from Upper Blue Nile.

Mr. A. L. Butler has again kindly named the birds for us. Human pathology has, however, claimed so much time that the study of the hæmatozoa of birds and the lower animals has perforce been abandoned to a large extent.

X Bodies in Human Blood*

Plate XXII., fig. 1

As noted on *page* 24 of our First Review Supplement these curious bodies had once been noted in the blood of a sick dog in Khartoum.

On November 23, 1910, I received the following report, accompanied by the blood films mentioned, from Dr. Nicola Mãloûf, of the Sudan Medical Department, one of the Medical Officers in Omdurman, who, when in Khartoum, had been diligent in his attendance at the laboratories.

"I am sending you 4 slides of blood from a patient under my care who is suffering from a very interesting disease, which I might call *Urticaria nocturna*. The disease is of 4 months standing; it appeared at Kamlin. The patient is a white lady about 18 years old, married, and is nursing a child, 1 year old; the child is very healthy. The case presents the following symptoms:—Every evening, about 7 p.m., flat, solid, more or less elevated wheals, irregular in size and shape begin to appear all over the body. These wheals disappear and others appear till the early morning, when the eruption begins to diminish, and, about sun-rise, nothing is left and the patient looks normal, like any other person. The eruption is accompanied by a severe itching and burning sensation; there is no fever and no disturbance of any of the systems of the body. The lady lived in Kamlin for the last two years.

Description of the case

"The slides are not fixed. Will you kindly examine them for anything you might think as a cause? If more slides are required or any other information is necessary, I will be very glad to help."

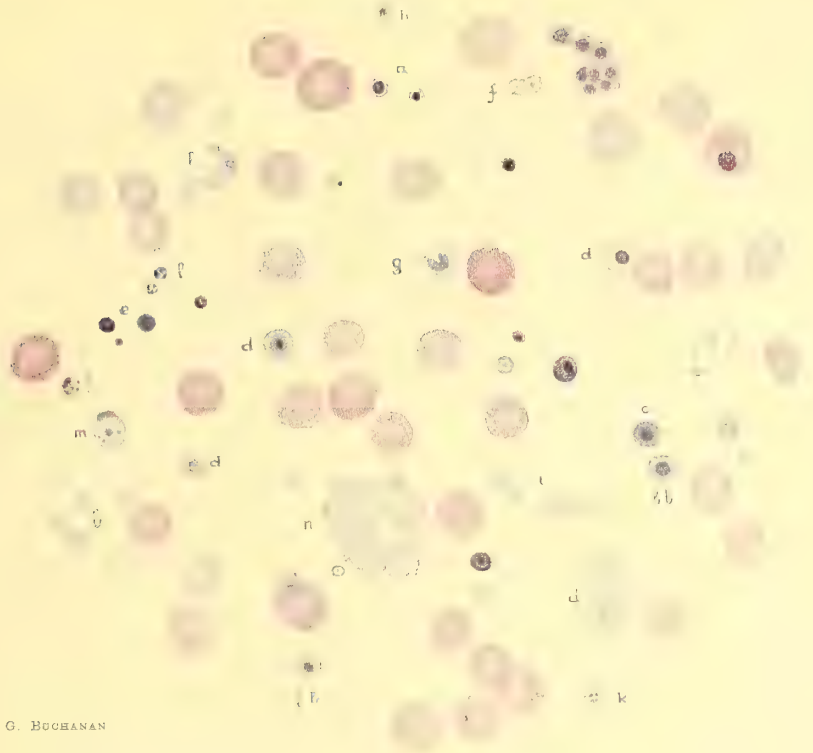
Enquiry elicited the fact that at no time had there been any fever.

In one of the films I found typical X bodies in considerable numbers, free and occasionally lying on the red cells. A fairly common form was the small body (*a*) which Horrocks and Howell[1] describe as being about one-eighth the size of a red corpuscle, spherical in form and consisting of a faint capsule with a circular centre staining deep

* Reproduced by kind permission of the Editor, from the *Lancet* of February 4, 1911.

[1] Horrocks, W. H., and Howell, H. A. L. (April, 1908), "X bodies found in the Blood of Human Beings and Animals." *Journal of the Royal Army Medical Corps*, Vol. X., No. 4.

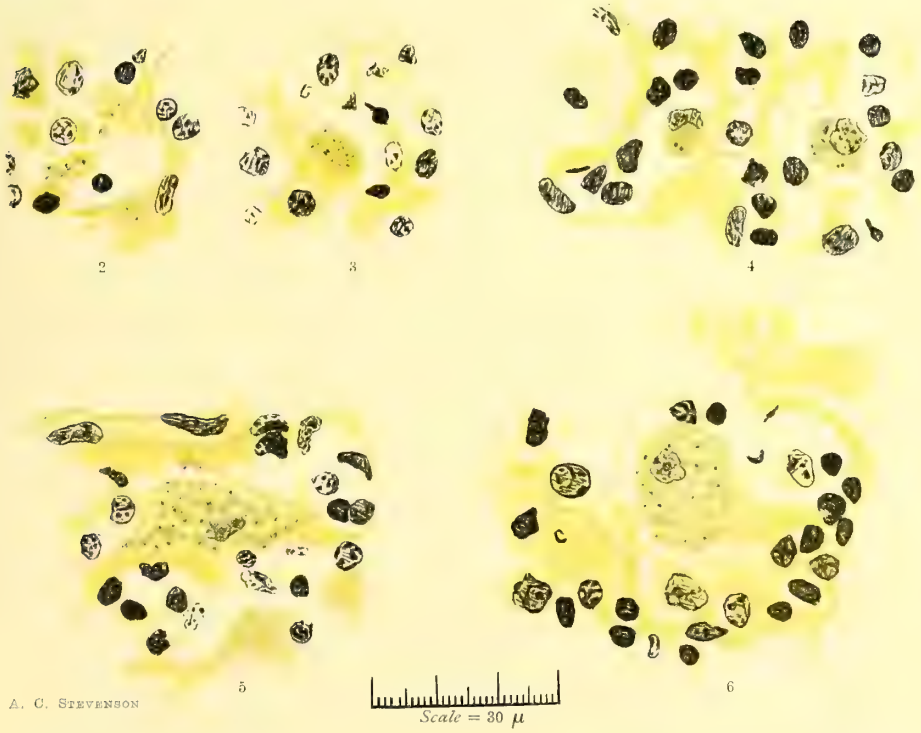
PLATE XXII



G. Buchanan

1. × Bodies in Human Blood from a Case of Intermittent Urticaria

Leishman Stain × 1000 *diam.*



A. C. Stevenson

Peculiar Bodies found in the Intestinal Lymphoid Follicules of an Egyptian

Drawings of portions of sections stained with Iron Hæmatoxylin and Orange

2 & 4. Small and medium-sized bodies in portions of cells

3. Large body lying apparently free in lymph space

5 & 6. Endothelial cells in lymph space packed with bodies of varying size

blue. The majority, however, had centres more of a pale lilac than a blue colour and contained faintly staining material between their capsules and their centres (*b*). What may be called large and intermediate forms (*bb*) of a similar type were present, and, in the former, the capsule was not always so defined, the appearance being rather that of a halo of more faintly staining substance surrounding a deeply stained spherical core (*c*). Very few forms with concentric rings (*d*) were observed and only one or two of the large "solid" coccus-like type (*e*) shown in the drawing A, which so well illustrates the paper by Horrocks and Howell. One did not see the type with an indented centre, crescent-shaped, and enclosing a small circular body of which they speak, but the forms, apparently in process of division, were present (*f*). I am not sure that these, especially when occurring in groups of three, do not really represent fused forms. Bodies which may be described as fragmented were encountered. Some of these have only a chip, as it were, broken from them or look like biscuits from which one has bitten out a piece (*g*). Distinctly oval forms were seen, usually of considerable size (*h*) and also a curious oblong appearance which it is difficult to describe but which is well shown in (*i*). There can be no doubt these forms consist of the same substance as the bodies. For convenience I will call them the oblong forms. Again, small forms measuring 3 μ in diameter were met with, one of which showed a segmentation of the central spot into four (*k*), another no dark centre, but dark spots at its periphery (*l*) and yet another a kind of inner capsule. Many of the smallest forms were homogeneous showing no dark centres whatever, while some of the larger forms with distinct cores showed granular material between their capsules and their centres. In this there were sometimes small blue spots or granules. Others again were irregular in shape, being what may be called "knobbed" bodies (*m*).

Types of "bodies" found

Like Horrocks and Howell, I found no trace of chromatin in these bodies, but I noticed that, lying free, there was a considerable amount of amorphous-looking material which took on a pale lilac stain like some of the smaller bodies. This was more plentiful in that part of the film where the bodies occurred, for I did not find them equally distributed throughout, and I could not help thinking that there was some connection between the bodies and this material, *i.e.* that they might represent portions of it which, owing to the action of the circulating blood, had assumed a spherical shape just as do fragments of macrophages. Still, it was difficult to account for the concentric rings on this hypothesis, and altogether I found it impossible to come to any conclusion regarding them. At the same time, further study convinced me that the smaller homogeneous bodies, at least those whose staining reactions and definite form readily distinguished them from blood plates, are in reality derived from the irregular amorphous masses. One can, as it were, see them budding and breaking off from the latter which themselves are very curious and interesting, containing, as they often do, an irregular central cavity and being furnished with edges which resemble the outline of a very rugged and indented sea-coast (*n*). The following are the measurements of the various forms of bodies found:—

No chromatin seen in the bodies

Spherical. These ranged from ·7μ to 5·25μ in diameter, 1·5μ was common and 2·25μ even more so, the majority being of this dimension; 3μ and 3·3μ were, however, not at all rare. Fused or dividing forms frequently measured 3μ each. This was also the size of some small "solid" coccus-like forms, four of which were found lying close together. One so-called oblong form measured 6·75μ in length by 3μ in breadth, and another, which lay end to end with it, 4·5μ by 3μ. It will be seen then that the bodies are pleomorphic although quite distinct from all the blood elements and readily recognisable as specific entities. As I only found them in one film out of the four sent, it occurred

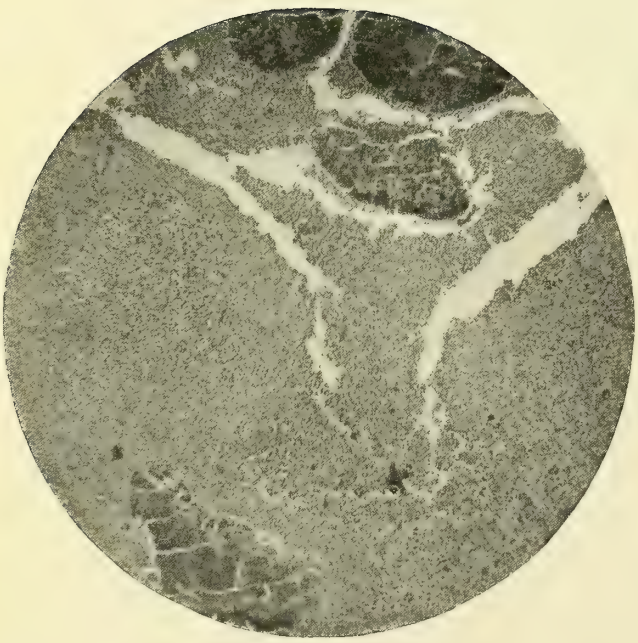
Measurements



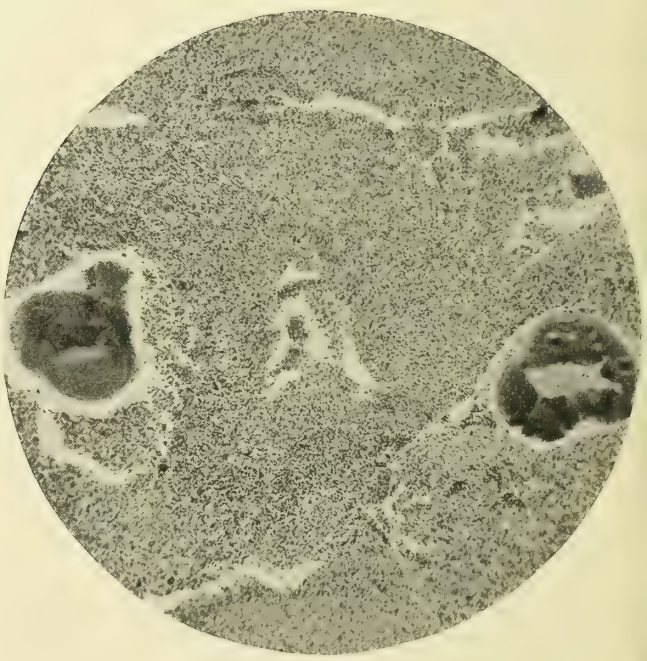
1



2



3



4

G. Buchanan

MYCETOMA

1. *Aspergillus bouffardi*—foot
2. Unclassified species of fungus grain—foot
3. *Madurella mycetoma*—large toe
4. *Indiella somaliensis*—hand

× 50 *diam.*

to me that their habitat might not be the blood but the skin, from which they may have been washed out by the first drop of blood. I had no means of telling which was the first film taken, but I noted that Horrocks and Howell, on several occasions, found the bodies in one slide out of several examined. On the other hand, this was by no means their invariable experience, while they once recovered the bodies from broth into which a piece of the liver of an ox or cow had been placed. This would appear effectually to dispose of the idea that they are derived from any part of the skin unless some contamination had occurred.* The same remark applies to their successful inoculations of rabbits. I asked Dr. Mãloûf to send me some more blood films and he forwarded ten, all unfortunately taken from one of the urticarial wheals, and numbered in the order in which they had been taken. In none of them were X bodies found. The previous blood films had been made from finger blood. I now sent Dr. Mãloûf two tubes of Nicolle's blood agar and several tubes containing the 1 per cent. sodium citrate solution employed by Horrocks and Howell, and requested him to inoculate these tubes with appropriate quantities of blood taken from the finger as at first. This he kindly did, and I incubated the inoculated media at or about 22° C. The tubes were examined daily for five days, care being taken to secure the blood sediment from the citrate tubes. Unfortunately, coccal or bacterial contamination, apparently of cutaneous origin, eventually made its appearance in every one of the tubes and at no time were any X bodies found. I tried hard to get the patient to visit the laboratories but in vain, and before Dr. Mãloûf could obtain other blood films or make other blood cultures she left for Kamlin on the Blue Nile. Hence, much to my regret, I was unable to pursue the case further or to confirm the findings of Horrocks and Howell in culture media or to repeat and extend their inoculation experiments.

Attempts at Culture

Although one cannot express any opinion on these curious bodies their occurrence in the Sudan is certainly interesting as is the fact that they were found in a case unassociated with fever but suffering from a recurrent or intermittent urticaria.†

Mycetoma

In the light of Brumpt's classification[1] we are now in a position to say something more definite regarding the varieties of mycetoma encountered in the Sudan. Plate XXIII. shows four species of fungus grain namely *Madurella mycetoma*, *Aspergillus bouffardi*, *Indiella somaliensis* and an unclassified species.

Four varieties

It is not intended to enter into any detailed account of these forms. Brumpt's excellent memoir deals fully with the subject, and the Sudan types, with one exception, appear to answer to his descriptions. In the case of the last named, the diagnosis is doubtful, for the specimen was received in spirit from Dr. Waterfield at Port Sudan and it was not possible to carry out cultivation experiments. The grains, however, were different to any hitherto seen, being of a rich brick-red colour, very hard and spherical in shape. Captain Archibald, who examined the specimen, found, on crushing the grains,

* *Note.* Mr. George Buchanan, however, drew my attention to the fact that some of the forms were present on the slide beyond what one may call the "commencement end" of the blood film. This rather suggests that they may have been derived from the surface of the skin, especially as, in all probability, the slide was pressed down upon the finger in order to take up the drop, and not merely allowed to come in contact with the latter.

† Since this paper was written, X bodies have been found by Major Ensor in blood films in a case of indefinite fever in the Military Hospital, Khartoum, and in smears of blood obtained by splenic puncture in a case of Kala-azar and sent to the Laboratories from Gallabat in the Kassala Province. A few were also encountered in a film of chick's blood made by a native attendant in the laboratories. None were found on his skin.

[1] Brumpt, E. (November 25, 1906), "Les Mycétomes." *Arch. de Parasit.*, Vol. X. No. 4, and in *Précis de Parasitologie.* Paris, 1910.



G. Buchanan

Fig. 109.—Mycetoma—*Indiella somaliensis*



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Fig. 110.—Mycetoma—*Indiella somaliensis*



Dr. Mălo͡e

Fig. 111.—Mycetoma—*Indiella somaliensis*

an appearance suggestive of Aspergillus infection, while on section, as will be seen (Plate XXIII., fig. 2) the grain does not answer very well to any of Brumpt's types. There has not been time for any detailed study of the specimen but it is worth recording as being possibly a species as yet unknown to Science.

Leprosy

Figs. 112 and 113 show a marked case of typical tuberculous leprosy in a young native woman. I took the photographs at Wau. The patient was under the care of Major Howard Ensor, D.S.O., R.A.M.C., and at a later period he found lepra bacilli in the nasal discharge and in scrapings from the lesions. The leonine appearance of the face, giving the impression of considerable age, is well marked.

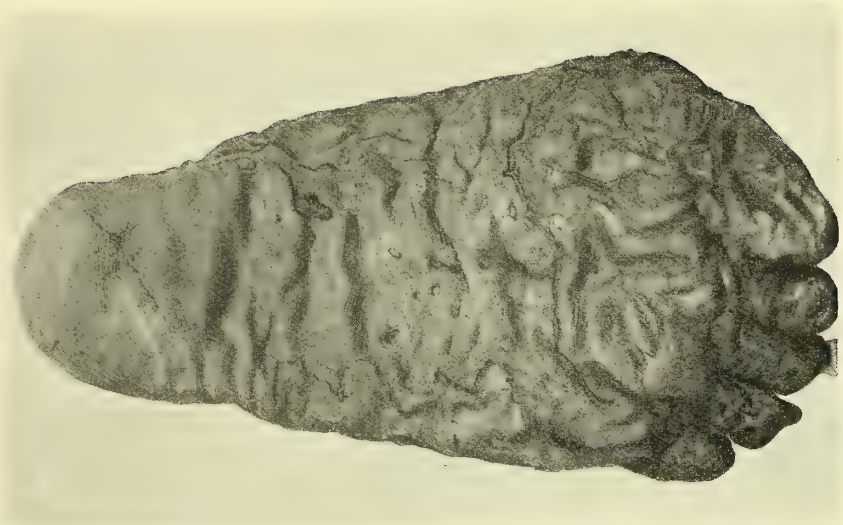


A. Balfour A. Balfour

Figs. 112-113.—Typical tuberculous leprosy in young native woman

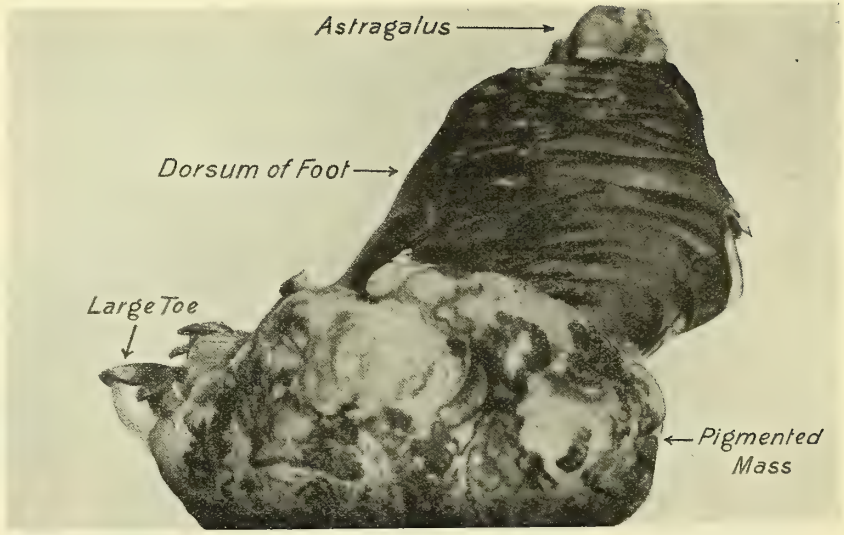
Dysentery

The only point to be noted under this heading is that it has been definitely proved that both amœbic and bacillary dysentery occur in the Sudan. The former is due to *Entamœba histolytica* (Schaudinn); the latter, in an interesting case occurring at Atbara, was proved by a marked agglutination reaction to be due to *B. shiga*. It is probable, therefore, that this bacillus is the most frequent cause of dysentery of this type in the Sudan which, however, requires further investigation.



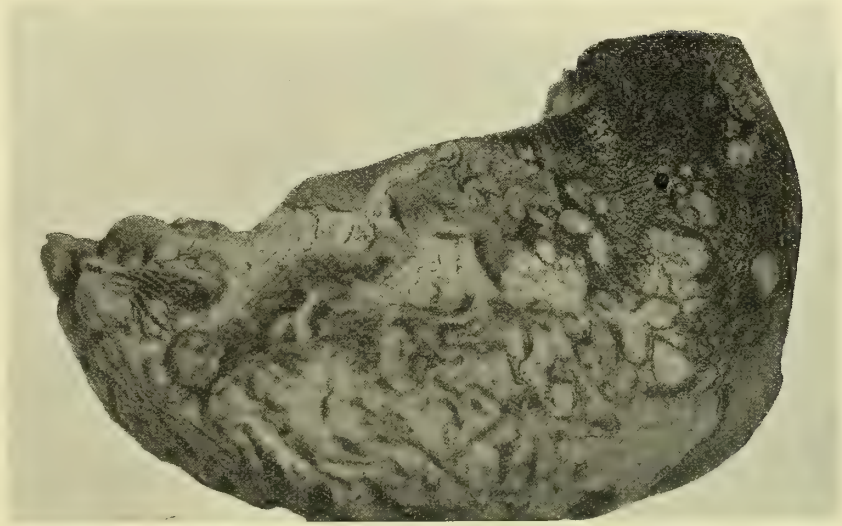
G. Buchanan

Fig. 114.—Mycetoma—*Aspergillus bouffardi;* showing plantar sinuses



G. Buchanan

Fig. 115.—Mycetoma—*Madurella mycetoma*



G. Buchanan

Fig. 116.—Mycetoma—Unclassified Species



G. Buchanan

Fig. 117.—Mycetoma—Fungoid form of *Aspergillus bouffardi*

Peculiar Bodies found in the Intestinal Lymphoid Follicles of an Egyptian

BY

A. C. Stevenson, M.B., M.R.C.S., L.R.C.P. (Lond.), D.P.H. (Cantab.)

(Plate XXII., figs. 2–6)

This plate is to illustrate peculiar bodies found in the solitary lymphoid follicles of the small intestine of a man, an Egyptian, who died suddenly in Khartoum. The tissue was sent home to me by Dr. Balfour on the chance of the case being one of coccidiosis of the intestine, the macroscopic appearance being practically the same as that seen in coccidiosis of the goat's intestine which we had observed shortly before, and which is described elsewhere (*vide page* 355).

On section the epithelial cells of the mucous membrane were found to be practically destroyed, only a few cells, and those degenerate, being seen in the depths of the glands. How much of this was due to post mortem changes I cannot estimate. In the sub-epithelial layers of the villi, there seems an excess of cellular elements which are either very degenerate or possibly parasitic. Stained by iron hæmatoxylin and orange the majority of these cells show an indefinite unstained nucleus with one or more small chromatin dots; the few remaining cells stain normally so far as the nucleus is concerned. If parasitic they are possibly amœbæ (? histolytica), but I am more inclined to think them degenerate tissue cells.

Position of the masses

At the periphery of the lymphoid follicles and at their base are the masses illustrated (Plate XXII., figs. 2 and 4). They are large cells, probably the endothelial cells of the lymph spaces, swollen up so as to occupy practically the whole lumen, and packed with small round and oval bodies of about 1–5μ in longest diameter. These small bodies, when stained as mentioned above, are greenish in colour, generally show a black dot in their substance and the larger of them are usually situated nearer the centre of the mass than the smaller. Larger bodies of the same staining reactions were also seen in cells but were few in number (Plate XXII., fig. 3). Stained by Giemsa, the stain being used in the same method as Twort's for *Entamœba coli*, the clusters of small bodies are a bluish-purple while the ordinary nuclei are greenish-blue and the rest of the tissue pink.

Bacilli were seen in only one or two sections stained by Twort's stain. They were scattered and of large size, resembling those seen in much post mortem tissue in Khartoum. Tubercle bacilli were examined for but not found.

What is the nature of these bodies? Are they parasitic or are they massed in the cells by phagocytosis? I do not think they can be remains of the dead cells in the sub-epithelial layers as they stain absolutely differently with Giemsa, but on the other hand their colour by iron hæmatoxylin and orange closely resembles that of degenerative blood corpuscles in cells.

I have not found any of the larger bodies in the Giemsa-stained sections, so cannot say what their staining reaction is, but their presence certainly might suggest a parasitic origin for the smaller.

Since writing the above, I have found some very similar masses of bodies in similar situations in the stomach of a mouse which was in apparently good health.

Routine Work

The following is a list of the various examinations conducted in the bacteriological laboratory since the last list was compiled for the Third Report.

The period of work covered is 2 years and 7 months.

(*a*) Morbid secretions and excretions	244
(*b*) Blood	714
(*c*) Bacteriological apart from (*a*)	603
(*d*) Parasites apart from (*a*) and (*b*)	1
(*e*) New growths	53
(*f*) Other pathological conditions	176
(*g*) Medico-legal	7

I do not think it is necessary to consider these in more detail beyond saying that the fact that helminths were almost invariably sent either to Professor Looss or to Dr. Leiper for determination in part explains the low figure under "Parasites." Few specimens have, however, been sent for diagnosis. The considerable increase as regards "New Growths" is due in part to the inclusion of botryomycosis under this heading. I do not think there has been any increase as regards malignant tumours.



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Fig. 118.—Floating Laboratory and S.W. *Culex*
Auxiliaries to the Wellcome Tropical Research Laboratories, Khartoum

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